Journal of Fiber Bioengineering & Informatics 5:1 (2012) 1–31
http://www.jfbi.org | doi:10.3993/jfbi03201201

Novel Biodegradable Functional Amino Acid-based Poly(ester amide) Biomaterials: Design, Synthesis, Property and Biomedical Applications

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Abstract

A new family of biodegradable and functional amino acid-based poly(ester amide)s (AA-PEAs) have been designed and synthesized as a new generation of biodegradable biomaterials for biomedical applications. This paper provides an overview of this new generation of biodegradable biomaterials, their design strategy, synthesis, characterization, unique biological property and eventual biomedical applications. AA-PEAs are synthesized from 3 basic building blocks: amino acids, fatty diols and fatty diacids. Due to the enormous variety of these 3 building blocks, a wide range of AA-PEAs could be tailor-designed for meeting specific clinical needs. These AA-PEAs have been engineered into a variety of physical forms ranging from 3D microporous hydrogels, melt-spun fibers, electrospun fibrous membranes, films and microspheres. All these AA-PEAs have shown 2 unique biological properties: support natural wound healing, and muted inflammatory response.

Keywords: Amino Acids; Poly(ester amide); Biodegradable; Inflammation; Synthesis; Biomaterials; Biomedical Applications; Fibrous Membrane; Electrospinning; Hydrogels; Microspheres; Fibers

1 Introduction

Absorbable or biodegradable polymeric-based biomaterials have received a lot of attention since their successful commercial launch as absorbable suture materials in early 1970s [1, 2, 3]. Those absorbable biomaterials are from aliphatic polyesters like Polyglycolic Acid (PGA), Poly-L-lactide (PLA), Poly-ε-caprolactone (PCL), poly-*p*-dioxanone and their copolymers. Absorbable polyesters have very good mechanical property and can be fabricated by thermal means into variety physical forms like fibers and micro- or nanospheres. Due to the presence of ester linkage in the polymer backbone, absorbable polyesters are subject to hydrolytic degradation via bulk degradation mode. Consequently, absorbable polyesters are moisture sensitive and require either rigorous inert dry environment or vacuum seal for long-term storage and better shelf life. In addition, due to their bulk hydrolytic degradation mode, the release profiles of the impregnated

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March 2012

drugs are difficult to achieve a liner fashion. Besides the original and commercially most successful use in wound closure devices, other biomedical applications of these absorbable polyesters have included drug carriers, components of orthopaedic, cardiovascular and dental devices, and scaffolds for tissue engineering.

One of the major drawbacks of these absorbable polyesters as biomaterials is the lack of functionality, i.e., no pendant functional groups for simple chemical modification or attachment of biologically active agents. Consequently, many functional absorbable aliphatic polyester derivatives have been developed and one unique approach is to introduce the natural amino acids into these absorbable polyester backbones [4-9]. The incorporation of natural amino acids will bring these aliphatic polyesters many new properties, such as functionality and charge property. One example of this approach is polyester-*b*-poly (amino acid) s, such as PLA-*b*-PLL and PCL-*b*-PLL [6, 7, 10]. This unique approach, however, has significantly altered the backbone structure of the original absorbable polyesters, and due to the tedious and complexity of the chemical reaction schemes, the yields of the final functional absorbable polyesters have been quite low and hence their acceptance for industrial use have been quite limited.

In late 1990s and early 2000s, a new family of functional amino acid-based poly (ester amide)s (AA-PEA) has been reported [11-13]. AA-PEAs differ from the traditional non-amino acid-based poly(ester amide)s that have been studied for many years [14-17]. Unlike the diamine-based non-amino acid PEAs, the amino acid-based PEAs derive their amide (or peptide) linkage from amino acids. As a result, the backbone chemical structure of AA-PEAs has both peptide and non-peptide bonds, and hence exhibit both proteins and non-protein properties, named as "pseudo-proteins". In this review, the basic design and synthesis strategies, the resulting chemical structure, property, fabrication/formulation and performance of the many generations of AA-PEAs will be given.

2 Basic Chemical Structure of Amino Acid-based Poly(ester amide)s and Their Design and Synthesis Strategies

The functional amino acid-based poly(ester amide)s are built from 3 basic non-toxic building blocks: amino acids, diols and diacids. Fig. 1 shows a generic chemical structure of the AA-PEA repeating unit:

As Fig. 1 shows, the 2 adjacent amino acid moieties per repeating unit are separated by both a diol (via 2 ester bonds) and diacid (via 2 peptide bonds) segments. Thus AA-PEAs are the same as polypeptides, except the sequence of peptide bonds are disrupted by ester bonds due to the

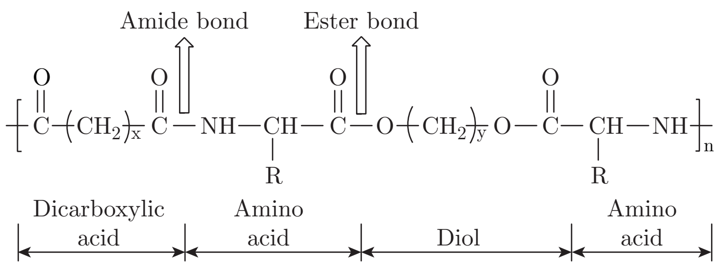


Fig. 1: General chemical repeating unit of a generic AA-PEA.

presence of diols and diacid segments.

This generic chemical repeating unit illustrates that there are many material parameters that we can use to design a variety of AA-PEAs, and these material parameters include: type of amino acids, type and length of fatty diacid (x) and fatty diol (y), and the location of amino acids. Up to now, a considerable variety of different amino acids have been used for the design and synthesis of AA-PEAs, and these amino acids include Phe, Arg, Lys, Leu, Gly, Ala, Ser, Val [11, 12, 13, 18-48]. Therefore, there are enormous varieties of AA-PEAs that could be tailor-designed for specific clinical uses – one of the major characteristics of AA-PEAs. In this review, many examples developed in my lab will be given to illustrate such a design strategy.

The general scheme of the synthesis of AA-PEA could be divided into three major steps: the synthesis of di-p-nitrophenyl ester of dicarboxylic acids as monomer (I); the synthesis of tetra-p-toluenesulfonic acid salts of bis (L-amino acid) α, ω-alkylene diesters as monomer (II); and the synthesis of AA-PEA (III) via a solution polycondensation of monomers (I) and (II). Fig. 2 shows the generic chemical structure of monomers I (Fig. 2 (A)) and II (Fig. 2 (B)) and their polycondensation into AA-PEA (III) (Fig. 2 (C)).

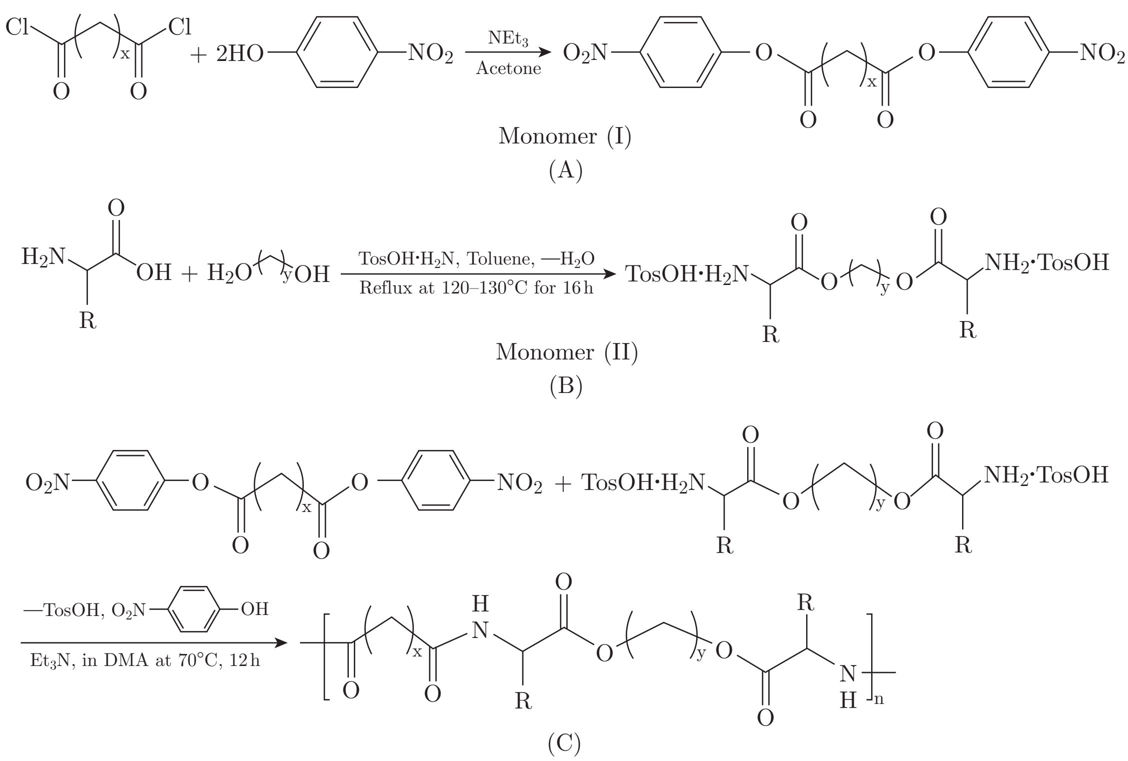


Fig. 2: (A) synthesis of di-p-nitrophenyl ester of dicarboxylic acids as monomer (I); (B) Synthesis of tetra-p-toluenesulfonic acid salts of bis (L-amino acid) α, ω-alkylene diesters as monomer (II); (C) Synthesis of AA-PEA via polycondensation of monomers (I) and (II)

A variety of advanced generations of this basic general synthesis of AA-PEAs has been reported recently. They include: the use of unsaturated diols or/and diacids [21-24, 29, 48] to synthesize unsaturated AA-PEA generations, the use of either polar amino acids like Lys, Arg, Ser or unsaturated >C=C< moiety in the backbone or pendant group to provide pendant functionalized AA-PEAs [11, 22, 25, 33-39, 48], and the different arrangement of these building blocks along the backbone [21, 22, 35, 36].

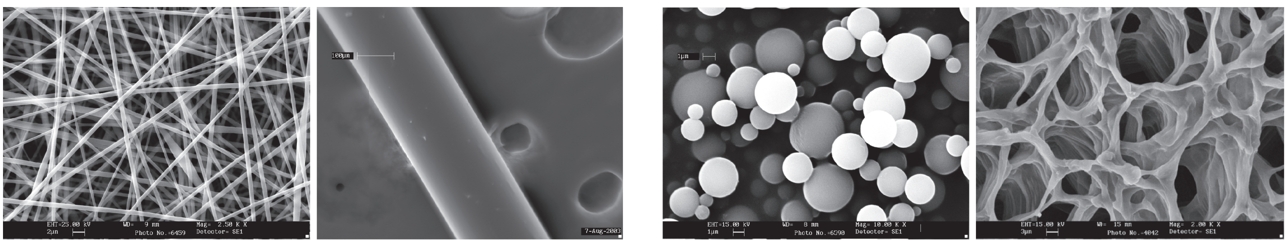
Among these different generations of AA-PEAs, hydrophobic non-polar amino acids like Phe, Leu, Val, Ala and Gly always result in neutral and water insoluble AA-PEAs, while ionic/polar amino acids like Lys, Arg, Ser could lead to either anionic or cationic AA-PEAs. Among those polar amino acids used in designing and synthesizing AA-PEAs, Lys and Arg are the most unique and studied because of their very unusual charge characteristics. For example, Arg has the highest isoelectric point 10.76 [24, 39, 44, 48] due to its strong basic guanidine group, and Lys has pI value of 9.59 due to its free amine groups, suggesting that AA-PEAs designed from these 2 particular amino acids could not only have the potential to be water soluble but also retain their charge characteristic over the most pH range. Because of the presence of 2 free amine groups in Lys, Lys-based PEAs could be designed to exhibit either anionic charge via its pendant –COOH group [11, 25] or cationic charge via its pendant $-NH_2$ group [32, 34, 37].

This strong cationic charge characteristics of both Arg-based or Lys-based PEAs have been shown to be able to condense anionic charged biopolymers like proteins and nucleic acids at a far lower cytotoxicity than commercial and experimental transfection agents like Lipofectamine 2000®, Superfect®, and PEI [39, 47, 48]. Furthermore, these cationic AA-PEAs have also been reported to show excellent cell attachment and proliferation for a variety of cells like human fibroblasts, bovine vascular endothelial cells, rat vascular smooth muscle cells [32, 34, 36, 39]. Finally, the cationic Arg-based PEAs have been show to have excellent cell membrane penetration capability, and have been extensively studied as gene transfection agents as described under 6. Biomedical Application section.

3 Fabrication of AA-PEAs Into Variety of Physical Forms

In order to utilize these new AA-PEAs, they must be able to be engineered to a variety of physical forms. Our lab has developed the fabrication technologies that can engineer AA-PEAs into electrospun nanofibrous membranes [28, 40, 49, 54], melt-spun fibers [48], micro and nanospheres [50], 3D microporous hydrogels [22, 29, 51, 52] and films [32]. Fig. 3 shows the images of these AA-PEA physical forms engineered. These physical forms of AA-PEAs have also been tried to delivery drugs and proteins (e.g., bFGF, IL-12, pactlitaxel, albumin, nitric oxide derivatives, gallium nitrate, antibiotics, biotin, dyes) via pre-loading, post-loading or chemical conjugation modes.

For examples, Phe-based PEA microspheres having a mean diameter diameter <1 μm with very narrow size distribution were obtained at a fair yield about 80% [50]. Paclitaxel loaded Phe-PEA microspheres showed near 100% drug loading efficiency, suggesting that AA-PEA microspheres



(A) (B)

Fig. 3: (A) Electrospun AA-PEA fibrous membrane (left) and melt-spun fiber (right); (B) AA-PEA microspheres (left) and 3D microporous hydrogel (right).

may have the potential for the injection administration of highly hydrophobic anticancer drugs. Research is in progress in the Chu's lab to engineering AA-PEA nanospheres, and the preliminary data indicate that the AA-PEA nanospheres of mean diameter around 158 nm with a very narrow size distribution could be fabricated.

Similarly, Phe-PEA electrospun fibrous membrane (fiber mean diameter about 640 nm in Fig. 3 (A)) that were pre-loaded with a nitroxyl radical, 4-amino-2.2.6.6-tetramethylpiperidine-1-oxy(4-amino-TEMPO), showed a linear release profile of 4-amino-TEMPO as a function of square root of time of release (Fig. 4) [28].

Not all AA-PEAs developed could be fabricated into 3D microporous hydrogel network, and the key requirement is that the AA-PEA macromolecules must have photo-reactive unsaturated >C=C< groups either in the backbone [21, 22, 29, 53] or as pendant group [35, 36, 51]. Although these AA-PEA hydrogels all exhibit 3D microporous network structure, there are considerable differences in terms of hydrogel morphology and pore size and shape due to the difference in the 3 basic building blocks used as well as the nature of the co-precursors. For example, the Phe-based PEAs show far more dense 3D microporous network structure than the Arg-based PEAs as shown in Fig. 5.

Besides the difference in 3D microporous network structure due to different AA-PEA precursors

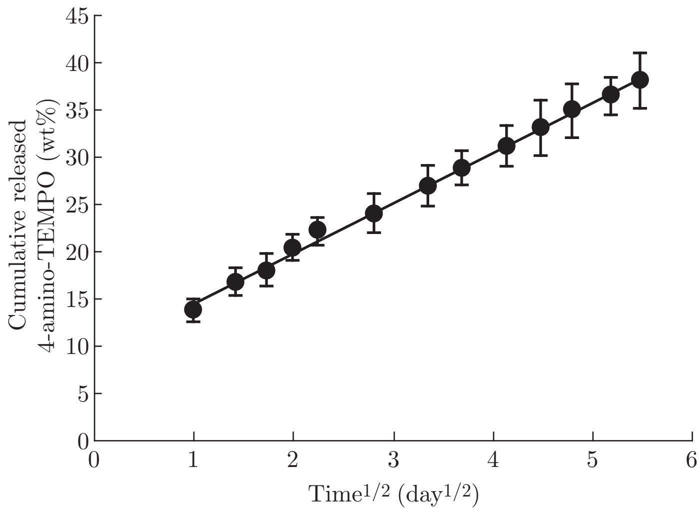


Fig. 4: Cumulative release of 4-amino-TEMPO from 640 nm 8-Phe-4 electrospun fibers in a pH 7.42 PBS medium at 37℃ as a function of square root of time. The 4-amino-TEMPO loading amount was 10.0 mg/g 8-Phe-4 [28].

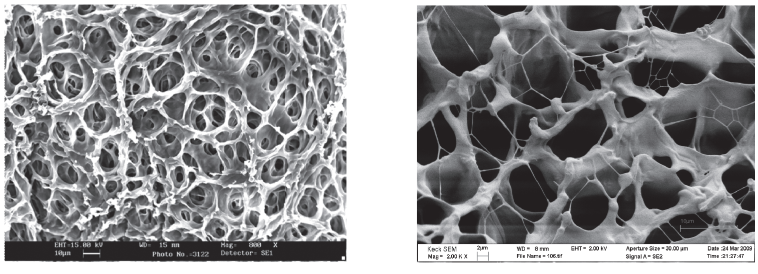


Fig. 5: AA-PEA hydrogels. Left - Phe-based PEA with polyethylene glycol diacrylate as the co-precursor (29); Right - Arg-based PEA with pluronic acid diacrylate as the co-precursor [39].

used, the Arg-based PEA hydrogels have cationic charge characteristic, while the Phe-based PEA hydrogels have no charge. As a result, it has been demonstrated that the cationic Arg-based PEA hydrogels exhibited very good cell biocompatibility, attachment and proliferation [39].

4 Enzymatic Biodegradation via Surface Erosion Mode

Unlike the hydrolytic bulk degradation mode of synthetic absorbable polyesters, due to the presence of amino acids, AA-PEAs have been shown to be enzymatically biodegraded via surface erosion mode [21, 22, 26-29, 35, 39, 48, 50-52]. This surface erosion mode of enzymatic biodegradation distinguishes AA-PEA from all absorbable polyesters. Fig. 6 (right pane) shows the *in vitro* α-chymotrypsin enzyme effect on the biodegradation of AA-PEA fibers as compared with the PBS control (left pane) up to 2 days. These AA-PEA fibers were completely biodegraded at the end of 2 days in α-chymotrypsin enzyme solution (right pane)

The enzymatic biodegradation of AA-PEAs is independent of their physical form. Fig. 7 shows the scanning electron microscopic interior morphology of a Phe-based PEA hydrogel with polyethylene glycol diacrylate as a co-precursor (FPBe-G37) after one (left) and 31 days (right) in a α-chymotrypsin (0.1 mg/mL) PBS medium at 37℃ [29].

The weight loss data of these Phe-based PEA hydrogels confirm the change of their morphology shown in Fig. 7 [29]. Fig. 8 shows such a weight loss data of a typical Phe-based PEA hydrogels with polyethylene glycol diacrylate as the co-precursor [53].

Fig. 6: *In vitro* enzyme hydrolysis of Phe-based PEA melt spun fibers at α-chymotryps enzyme concentration of 0.1 mg/ml in 10 ml of PBS buffer. Left 2 bottles: in pure PBS for 1 (far left) and 2 days. Right 2 bottles: in enzyme/PBS solution for 1 and 2 (far right) days. No visible trace of AA-PEA fibers could be found at the end of the 2 days immersion in enzyme/PBS solution (far right).

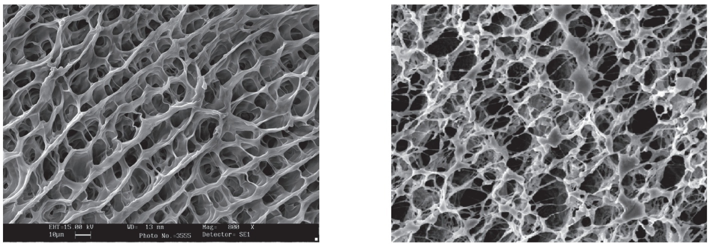


Fig. 7: SEM interior morphology of Phe-based PEA hydrogel (FPBe-G37) with polyethylene glycol diacrylate as the co-precursor [29]. The hydrogels were immersed in a α-chymotrypsin (0.1 mg/mL) PBS medium at 37℃ for 1 day (left) and 31 days (right).

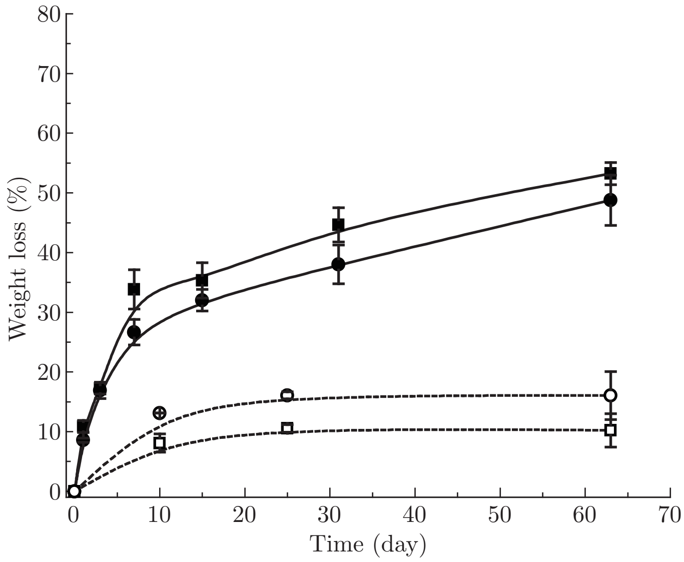


Fig. 8: The effect of precursors feed ratio of FPBe-G hydrogel on its biodegradation. (α-chymotrypsin concentration 0.1 mg/mL). Open symbol – pure PBS mediu; Solid symbol – enzyme/PBS medium. Circle - FPBe-G28 hydrogel; Square – FPBe-G37 hydrogel. G37 hydrogel had more Phe-PEA precursor than G28 hydrogel [53].

The enzymatic catalytic biodegradation effect on AA-PEA hydrogels is obvious in Fig. 8 as the weight loss of these hydrogels kept increasing with time in an enzyme medium (solid symbol), while the weight loss of the same hydrogels in a pure PBS medium (open sympbol) reached a constant level far below the enzyme case.

The reported enzymatic biodegradation data of AA-PEAs also show that an increase in the hydrophobicity of AA-PEAs (via longer methylene groups in diols or/and dicarboxylic acid segments) leads to a faster enzyme-catalyzed biodegradation [12, 27]. In addition to the hydrophobicity factor to interpret this relationship between the length of methylene groups in diols/diacid segments and enzyme-catalyzed biodegradation rate, the lack of intermolecular hydrogen bonds resulting from misalignment of the adjacent AA-PEA macromolecules was also suggested as a possible cause behind such a relationship.

All the reported enzymatic biodegradation data of AA-PEAs clearly illustrate that AA-PEAs are enzymatic biodegradable via surface erosion mode. This mode of biodegradation is more favorable than the bulk hydrolytic degradation for several reasons. First, a layer-by-layer erosion mode like peeling an onion could have a far more uniform release of impregnated drugs within the carrier matrix than the bulk mode, i.e., close to an ideal 1st order drug release profile. Second, biodegradable implants with a surface erosion mode could largely eliminate the release of large fragments of the implants that could break away during a biodegradation process, i.e., maintaining structural integrity longer and better. This is of particularly advantageous in the case of cardiovascular or any duct repair as those large fragments from bulk degradation of an implant could block the passage of smaller vessels/ducts. Thirdly, the shelf-life of those medical devices made from enzymatic biodegradable biomaterials like AA-PEAs should be far more stable and longer than the moisture-sensitive hydrolytically degradable absorbable polyester biomaterials.

A preliminary *in vivo* biodegradation study of selected AA-PEA (4-Phe-4) films in dorsum of rats with and without lipase-impregnation showed that those AA-PEAs were completely absorbed within 1–2 months post-implantation (for the lipase-impregnatedones) and 3–6 months (for the lipase-free ones) [27]. In a separated *in vivo* study in a porcine coronary artery model as described later in the Section 6 Biomedical Applications, it was also found that a Leu-Lys based PEA copolymer as the coating of a Genic stent had 70% AA-PEA mass remaining after one month postimplantation, an indication of an excellent slow and sustained-release drug delivery vehicle.

5 Biological Property

AA-PEAs distinguish themselves from commercially available absorbable polyesters in many unique biological properties that have been reported *in vitro* and *in vivo*. These unique biological properties range from blood biocompatibility, cell adhesion, attachment, and proliferation, support natural wound healing and muted foreign-body inflammatory response.

5.1 Blood Biocompatibility

In a reported *in vitro* study of blood and cellular responses to AA-PEAs, ATP release from the activated platelet adhered onto substrates was used to assess pro-thrombotic potential [38]. As shown in Fig. 9, AA-PEA-based polymers and a FDA-approved absorbable polyester (PLGA) had a similar ATP stimulating effect when thrombin served as the positive control. The amounts of ATP release from the AA-PEA copolymer (PEA-Ac.Bz) was approximately 50% of that of thrombin, while the nitric oxide derivative conjugated AA-PEA (PEA.Ac.TEMPO) induced about 35% of that of thrombin, i.e., about 15% lower platelet activation than PLGA and PEA-Ac.Bz [38]. Poly-n-butylmethacrylate (PBMA), a commercial FDA-approved coating material for commercial drug-eluting stents used by Johnson and Johnson and Boston Scientific, stimulated the lowest level of platelet activation of all of the polymers tested. Overall, all these polymers showed much lower ATP than the thrombin control.

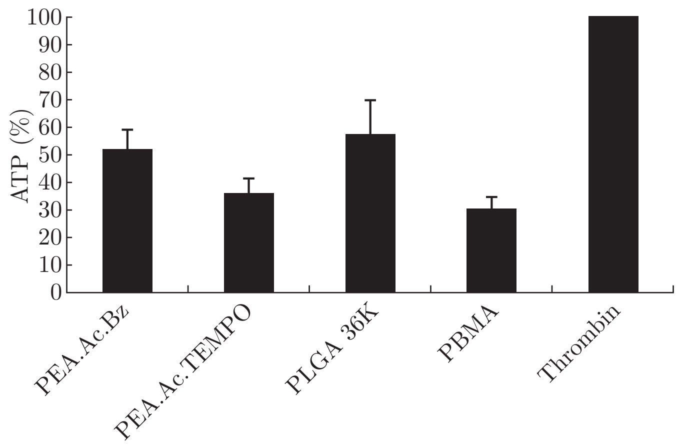


Fig. 9: Activation of platelets as expressed by ATP production in the presence of AA-PEA and other polymers. Results are expressed as percentage of activation by thrombin ± SEM (n=5) [38].

5.2 Cell Biocompatibility

Several *in vitro* cell culture studies using rat vascular smooth muscle cells, bovine aortic endothelial cells, human fibroblasts all indicate that AA-PEAs in a variety of physical forms (liquid, film, hydrogel) showed cell viability close to blank control and were significantly better than commercial polymers and transfection agents. Figs. 10 and 11 shows such cell viability and cell death data from the Arg-based PEA (Arg-PEA) [39, 47]. Over different types of Arg-PEAs and their concentration range, Arg-PEAs showed considerably higher cell viability and lower cell death than a commercial transfection agent, Superfect®, and the levels were close to blank control.

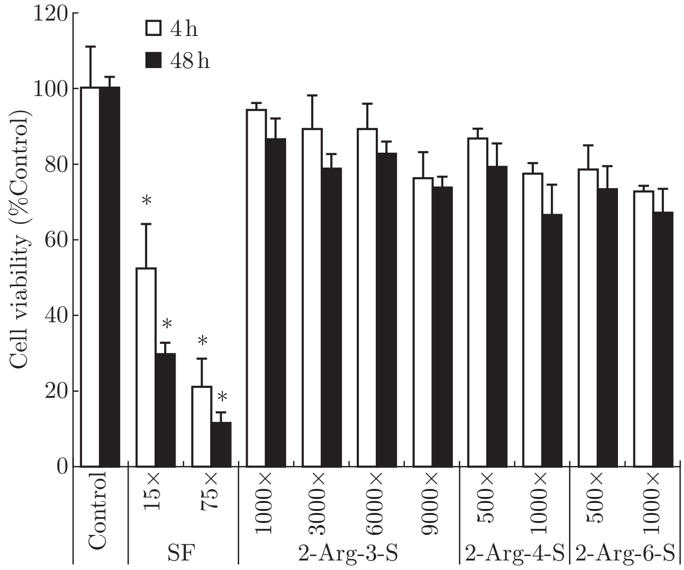


Fig. 10: Rat aortic vascular smooth cell viability data upon exposure to different types of Arg-PEA at different polymer concentrations. Blank and commercial Superfect® (SF) transfection agent are the controls [39, 47].

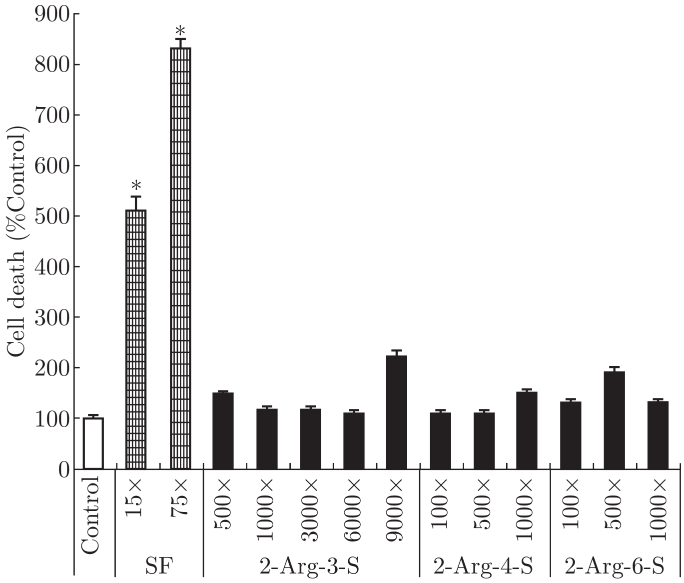


Fig. 11: Rat aortic vascular smooth cell death data upon exposure to different types of Arg-PEA at different polymer concentrations. Blank and commercial Superfect® (SF) transfection agent are the controls [39, 47].

These quantitative cell data were also confirmed in the qualitative rat aortic smooth muscle A10 cell morphological data shown in Fig. 12.

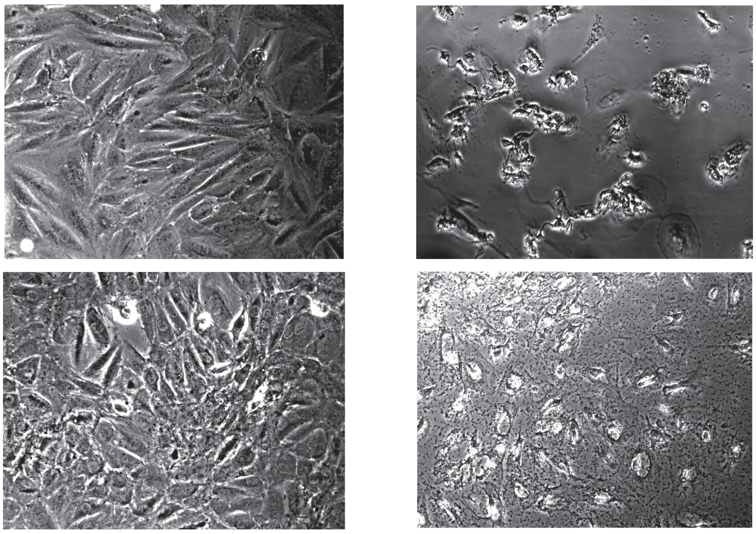


Fig. 12: Rat aortic smooth muscle cell (SMC) morphology after 24 hrs exposure to different transfection agents including Arg-PEA at 1,500 μg (top left), Superfect® at 15 μg (top right). The blank control cell medium only (bottom left), and PEI at 15 μg (bottom right).

The excellent aortic Smooth Muscle Cell (SMC) biocompatibility of AA-PEA is also evident in other types of cells including human fibroblast primary cells, normal human epidermal keratinocytes, human coronary artery endothelial cells, and bovine vascular endothelial cells as well as different physical forms of the AA-PEAs [33, 34, 36, 38, 39, 47, 54].

As shown in Fig. 13 [39], human fibroblast primary cells after 48 hr seeding on a cationic Arg-based Arg-PEA hydrogel surface (right) show fibroblast cell morphology very close to the tissue culture well blank control (left).

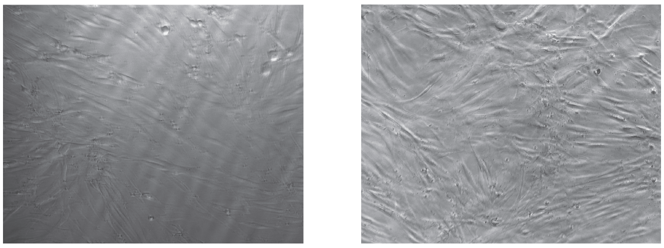


Fig. 13: Human fibroblast primary cells cultured on cationic Arg-based PEA hydrogel surface (right). The blank control is tissue culture well (left). Duration of cell seeding – 48 hrs [39]

Fig. 14 shows the bovine aortic endothelial cell (BAEC) adhesion and proliferation on a newer generation cationic Lys-based PEA substrate (8-Phe-4-Lys, left). Gelatin coating on a glass coverslip served as the control (right). The immunostaining data (Fig. 18) indicates that vinculin protein expression (green stain), an indication of focal adhesion, was as ample in the BAEC cultured on the AA-PEA substrate (left) as on the gelatin-coated glass coverslip substrate (right).

These qualitative BAEC data in Fig. 14 were further confirmed quantitatively as shown in Fig. 15 [34]. The proliferation data of BAEC on a newer generation cationic Lys-based PEA

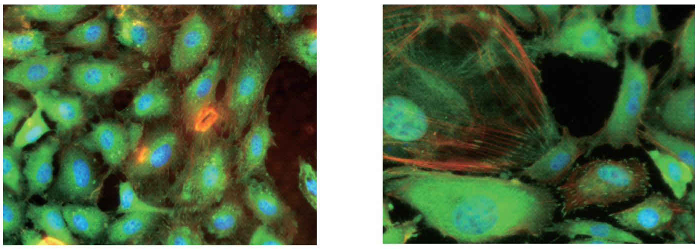


Fig. 14: Immunostaining of bovine aortic endothelial cells cultured on cationic Lys-based PEA substrate (left). Gelatin coated glass coverslip (right) served as the control. Vinculin protein was stained in green.

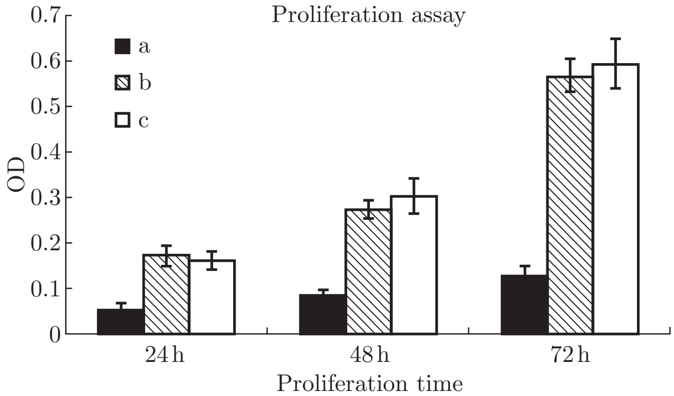


Fig. 15: Proliferation assay of bovine aortic endothelial cell on (a) Blank tissue culture control (without any treatment); (b) Gelatin coated tissue culture plate; (c) AA-PEA (cationic 8-Phe-4-Lys) over a period of 72 hrs [34].

substrate (8-Phe-4-Lys) shown in Fig. 15 [34] indicate that both AA-PEA and gelatin group showed very similar high proliferation rate, and this particular member of the AA-PEA family had a slightly higher mean cell proliferation data than the gelatin group. The support of cell attachment and proliferation by AA-PEA is not limited to cationic members of the AA-PEA family. When human coronary artery endothelial and smooth muscle cells were used on a neutral Phe-based PEA substrate, the cell proliferation on this neutral Phe-based PEA was 4 fold higher than on non-biodegradable polymers like poly(n-butyl methacrylate)/polyethylene vinyl acetate copolymer [38].

In a reported study from the Chu's lab [54], Normal Human Epidermal Keratinocytes (NHEK) were used to determine the capability of the Phe-based PEA electrospun fibrous membranes to support the NHEK growth over a period of 7 days *in vitro*. Two different live-cell assays, Calcein-AM and Alamar Blue, were used to help determine the amount of viable NHEK cells attached to the Phe-based PEA fibrous membrane scaffolds. Fig. 16 [54] shows the fluorescent confocal microscopic images of the Calcein-AM assay of NHEK (bright green) on Phe-based PEA fibrous membranes after 1 and 7 days culture. These *in vitro* NHEK data suggest that AA-PEA electrospun fibrous membranes could also support the proliferation of human epidermal keratinocytes.

5.3 *In Vitro* and *in Vivo* Inflammatory Response

Inflammation after injury is a double-edged sword. It is absolutely necessary to initiate an inflammatory response to resolve host infections or initiate wound repair. Yet, if uncontrolled,

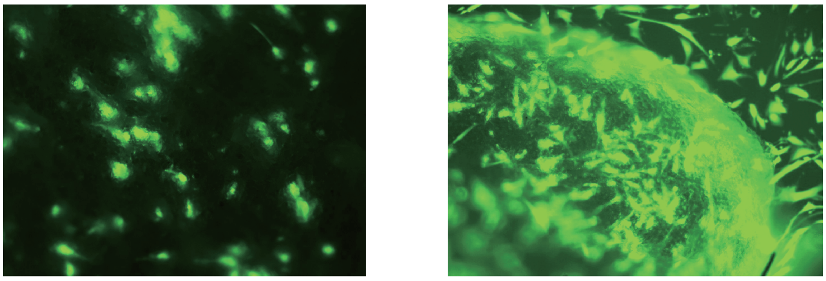


Fig. 16: Fluorescent confocal microscopic images of the Calcein-AM assay of NHEK (bright green) on Phe-based PEA fibrous membranes after 1 (left) and 7 days (right) culture [54]

particularly when foreign-body induced, it can lead to slower healing, tissue damage, loss of function, or in extreme cases, death. Controlling the inflammatory response to implantable or topical bioengineered medical devices is an important clinical challenge that complicates the use of biomaterials [55, 56], particularly in impaired wounds like diabetic or thermal injury. In thermal injury, there is often a super robust inflammatory response that destroys the necessary balance between inflammation and repair. This leads not only to local disrepair, but also to systemic illness, and in the most extreme cases, death. Hence biomaterials that do not add to the inflammatory response will likely result in both improved local wound healing and less systemic injury.

In their review of growth factor release from tissue engineered scaffolding, Whitaker et al. addressed the importance of inflammation, especially the foreign-body induced inflammation from the scaffold biomaterials [56]. It is important to recognize that all FDA-approved biomaterials and implants/medical devices made from biomaterials havel elicited foreign-body inflammatory response, and the issue is the level and duration of this foreign-body induced inflammatory response.

One of the most unique biological properties of the newly developed AA-PEA biomaterials

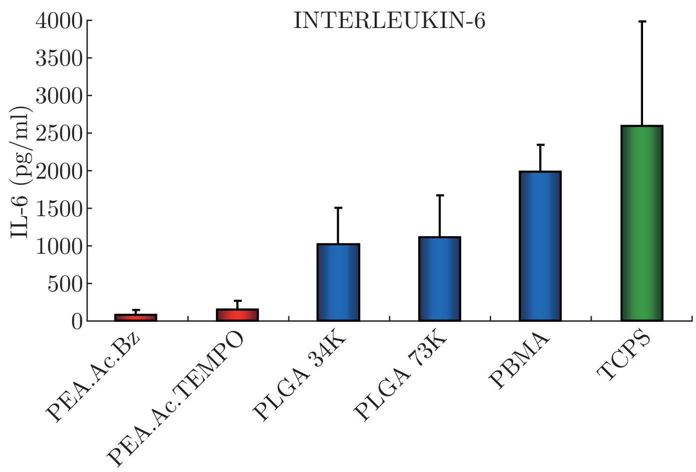


Fig. 17: IL-6 pro-inflammatory cytokine release upon 24 hr *in vitro* human peripheral blood monocyte culture on AA-PEA and other absorbable and non-absorbable polymers. 34K and 73K are molecular weights of the corresponding absorbable PLGA copolymers. PBMA: poly-n-butyl methacrylate, served as a commercial non-absorbable biomaterial control. TCPS (Tissue Culture-treated Polystyrene) served as the control [38].

is their muted inflammatory response as demonstrated both *in vitro* and *in vivo* [32, 38, 39, 40, 57]. As shown in Fig. 17 [38], human peripheral blood monocytes cultured on two types of AA-PEA (PEA-Ac-Bz and PEA-Ac-TEMPO) secreted over 5 fold less IL-6 pleiotropic pro-inflammatory cytokine release over 24 hrs than the classical absorbable polyglycolide/lactide copolymers (50/50 molar ratio), PBMA (poly-n-butyl methacrylate), and Tissue Culture-treated Polystyrene (TCPS). IL-6 can increase macrophage cytotoxic activity. PBMA is currently used by Johnson and Johnson and Boston Scientific as their non-biodegradable coating for their commercial drug-eluting stents to reduce restenosis problem associated with balloon angioplasty procedures.

A similar pattern of release of IL-1β, a potent pro-inflammatory cytokine, on monocyte cultured on the same AA-PEA biomaterials as those in Fig. 20 is shown in Fig. 18 [38]. The amounts of IL-1β released by monocytes on AA-PEA were 1/4 to 1/2 of the FDA-approved absorbable PLGA and non-absorbable PBMA biomaterials. It is important to know that IL-1β pro-inflammatory cytokine can also increase the surface thrombogenicity of endothelium.

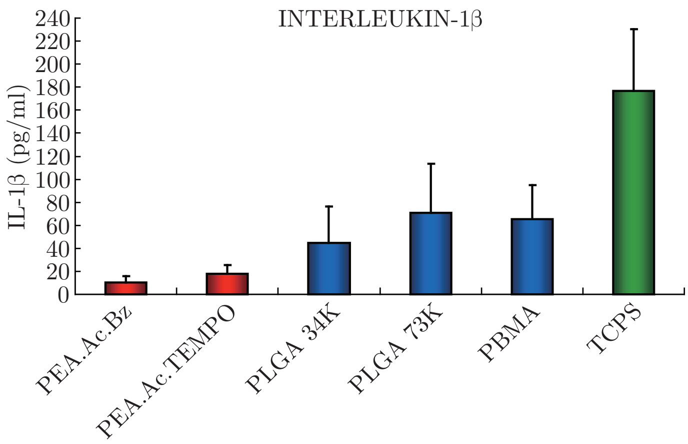


Fig. 18: IL-1β pro-inflammatory cytokine release upon 24 hr *in vitro* human peripheral blood monocyte culture on PEA and other absorbable and non-absorbable polymers. 34K and 73K are molecular weights of the corresponding absorbable PLGA. PBMA: poly-n-butyl methacrylate, served as a commercial non-absorbable biomaterial control. TCPS (Tissue Culture-treated Polystyrene) served as the control [38].

In a yet published study of several newer generations of AA-PEAs [39], Wu et al. have shown that these newer AA-PEAs themselves exhibited muted inflammatory response as evident by the very similar level of TNF-α production (by mouse J774 macrophages) as in the absence of any biomaterial control. Wu et al. also demonstrated that when a FDA-approved biomaterial, i.e., absorbable Poly-ε-caprolactone (PCL), was chemically coupled with these new AA-PEAs, the resulting hybrid exhibited lower TNF-α production than the pure FDA-approved PCL.

These *in vitro* inflammatory data show that AA-PEAs not only could have a muted inflammatory response themselves but also could reduce the inflammatory response of other FDA-approved biomaterials when they are chemically coupled with AA-PEAs. It is unclear what the fundamental mechanisms are behind this muted inflammatory response of AA-PEAs. Chu has suggested that the disruption of the peptide linkage in AA-PEA backbone macromolecules by non-amino acid building blocks (i.e., diols and diacids) may trick the surrounding living tissues that AA-PEAs are individual amino acids rather than foreign polymeric biomaterials of proteins or non-protein nature.

This unusual biological property of AA-PEAs (i.e., muted inflammatory response) could be their most important characteristic for their applications in the biomedical fields, particularly the coating of existing medical devices and implants for significantly improving their *in vivo* performance by reducing undesirable foreign-body induced inflammatory response. As described in the section of biomedical applications later (Section 6), many published and unpublished studies like coating for drug-eluting stents and suture devices have illustrated the enormous impact of having muted inflammatory biomaterials like AA-PEAs.

The *in vitro* low inflammatory data of AA-PEA described above are further confirmed in an *in vivo* porcine coronary artery model of AA-PEA coated stent [57]. Fig. 19 shows a Phe-based PEA coated metallic stent that was used for the porcine coronary artery model study [57].

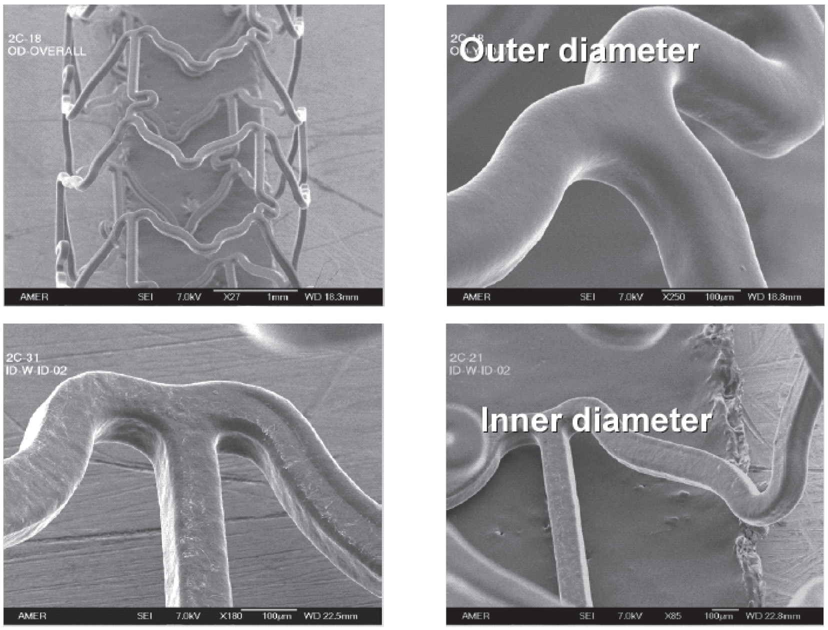


Fig. 19: Phe-based PEA coated Genic 316L stainless steel stent used in the porcine coronary artery model study [57].

After 28 days post-implantation, the AA-PEA copolymer-coated 316L stainless steel stent (Genic stent) exhibited statistically similar inflammatory score as the bare stent control (1.18 ± 0.38 of AA-PEA coated stent vs. 1.11 ± 0.32 of a bare stent control). There was no statistical difference in % area of stenosis and neointimal hyperplasia between the AA-PEA-coated and bare stents either. Their biocompatibility data in an *in vivo* porcine model suggest that these new biodegradable AA-PEA copolymers are as biocompatible as bare stents under a dynamic blood vessel condition [57]. As described later in the Section 6 Biomedical Applications, the AA-PEA-coated Genic stent in a multicenter human trial study also showed a much lower level of inflammatory response than a bare stent at 6 weeks postimplantation.

The *in vivo* muted inflammatory response of the AA-PEA biomaterials was also in *in vivo* implantation study of Phe-based PEA biodegradable fibers in rat gluteal muscle tissue up to 42 days as shown in Fig. 20.

The Phe-based PEA fiber showed far lower inflammatory response than a commercial absorbable suture, Monocryl from Ethicon. The tissue reaction score of the Phe-based PEA fibers was only 1/3 of the Monocryl suture fiber, and the Phe-based fiber site showed far better wound healing than the Monocryl site.

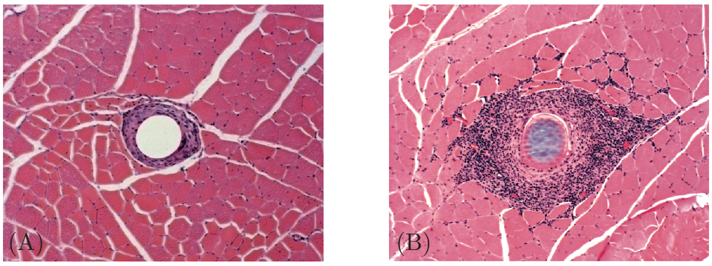


Fig. 20: Histopathology images of (A) a Phe-based PEA fiber and (B) 2/0 size Monocryl absorbable suture implanted in rat gluteal muscle for 42 days.

6 Biomedical Applications

This new family of synthetic biodegradable amino acid-based poly(ester amide)s has been studied for a wide range of biomedical applications, such as drug-eluting stents, wound dressing for treating burns, drug-delivery vehicles, vascular patch, scaffolds for tissue engineering, DNA carrier for gene transfection. In the following, several selected biomedical applications will be used for such an illustration.

6.1 As Drug-eluting Dressing for Burn Treatment – an *in vivo* Porcine Model Study

In the United States 40,000 people each year are hospitalized for burn treatment, and 5 million people each year suffer from non-healing wounds [58, 59]. Wound treatment is a significant burden on the American healthcare system, with yearly expenditures over $1.7 billion [60]. When considering the cost to society in lost workdays and productivity, management of these acute and chronic wounds exceeds $20 billion dollars annually [61].

For the past thirty year researchers have been searching for better methods to accelerate wound repair and improve the quality of the healed wound [62, 63]. The most recent approach involves bioengineered wound dressings that allow for the diffusion of pharmacologically active components into the wound [64].

In a simulated 2^{nd} degree burn (i.e., partial thickness) on a porcine model, Chu et al. used electrospun Phe-based PEA fibrous membranes (Fig. 3 (A), left) with or without the pharmacologic agent Gallium Nitrate (GN) were tested for safety and efficacy with a commercial DuoDerm® wound dressing as a control [40]. Fig. 21 shows the experimental wound model.

One of the most striking findings from this *in vivo* burn trial was the accelerated wound healing in the AA-PEA treated burn sites. For example, at the end of the 2 days postoperation, the AA-PEA-GN sites had already achieved a complete wound closure, whereas the control sites had not achieved complete wound closure and required re-dressing of test material. The control DuoDerm-treated burn site, however, caught up the AA-PEA-GN site at the 6^{th} day postoperation, and no visual difference could be detected.

Besides the much accelerated wound healing rate at the AA-PEA-GN treated burn site, the histology data showed that, at the end of the 9^{th} day postoperation, the regenerated skin tissue under the AA-PEA-GN burn site exhibited the irregular Rete ridge formation at the interface between epidermal and dermal layers (Fig. 22 (A)), while the control DuoDerm treated burn site

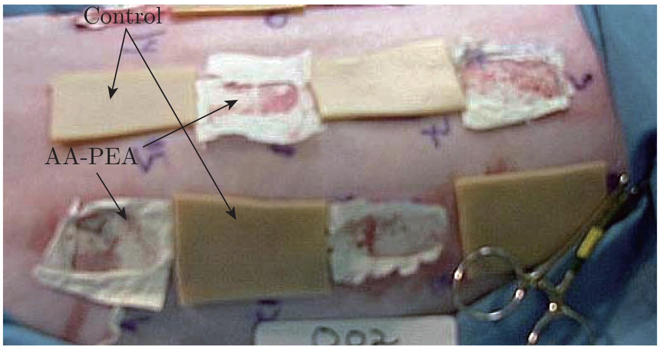


Fig. 21: Experimental setup for the coverage of 2$^{\text{nd}}$ degree burn sites by either gallium nitrate eluting AA-PEA electrospun fibrous membranes or a commercial DuoDerm wound dressing on a porcine model [40].

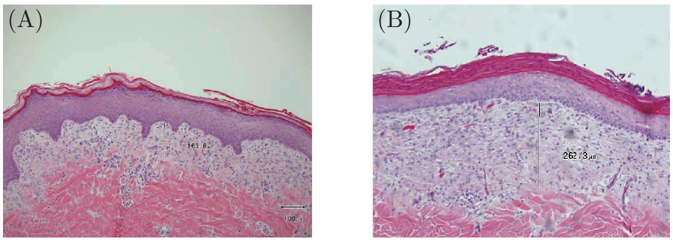


Fig. 22: H&E stain of the biopsy sample retrieved from the 9th day postoperation in a porcine burn model. (A) treated by AA-PEA-GN; (B) treated by a commercial DuoDerm wound dressing control. Note the Rete ridge formation in the regenerated skin tissue treated by AA-PEA-GN [40].

didn't show such irregular Rete ridge formation (Fig. 22 (B)).

It is important to know that a normal skin always exhibit such a Rete ridge formation. The much slower rate of Rete ridge formation under the commercial Duoderm dressing control is consistent with its slower rate of wound healing observed visually.

Another unique histological observation of the regenerated skin tissue under the AA-PEA-GN fibrous membranes was the much smaller fibrosis tissue formation and dermal proliferation than the commercial DuoDerm control at the end of 27 days postoperation (Fig. 23).

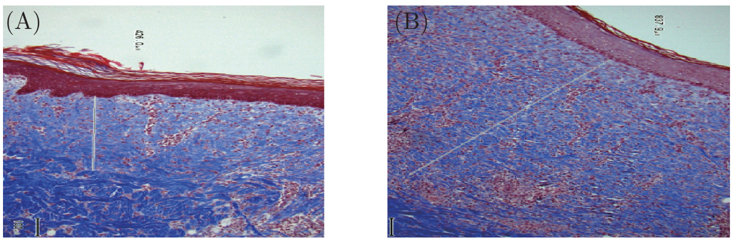


Fig. 23: Organized fibrosis of the regenerated skin after 27 days postoperation. (A) under AA-PEA-GN of fibrosis thickness ranging from 72-426 µm; (B) under commercial DuoDerm® control of fibrosis thickness ranging from 97-838 µm [40].

6.2 As Vascular Patch – an *in vivo* Rat Aortic Patch Model Study

All existing commercial vascular patches are made of synthetic non-biodegradable vascular fabrics like poly(ethylene terephthalate) polyester and poly(tetrafluoroethylene) [65]. Although these non-biodegradable fibrous polymers are adequate, due to their non-biodegradability, they elicit permanent foreign-body reaction.

The availability of biodegradable AA-PEA permits us to conduct a preliminary trial of AA-PEA based vascular patch for assessing the potential of AA-PEA biomaterials in cardiovascular reconstructive surgery.

A Phe-based PEA having impregnated with a Nitric Oxide Derivative (NOD) (4-amino-2.2.6.6-tetramethylpiperidine-1-oxy) was first fabricated into a tubular form via electrospinning method [66]. A sample of this NOD-eluting Phe-PEA tubular fibrous graft is shown in Fig. 24. This NOD-eluting Phe-PEA tubular vascular graft was shaped into a 5 mm diameter patch (Fig. 25) and then implanted in the 5 mm long defect created on a carotid artery of a male Sprague-Dawley rats (BW 400-450 g), and the wound was closed by 3/0 size Vicryl suture. A FDA-approved absorbable polyester (poly-ε-caprolactone, PCL) electrospun fibrous patch was used as a control.

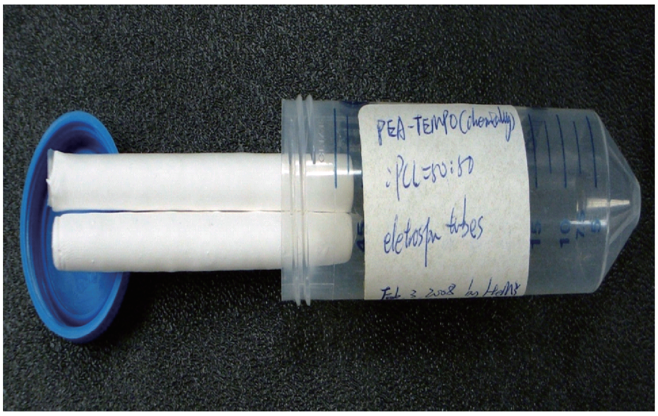


Fig. 24: Electrospun NOD-eluting Phe-based PEA tubular fibrous graft.

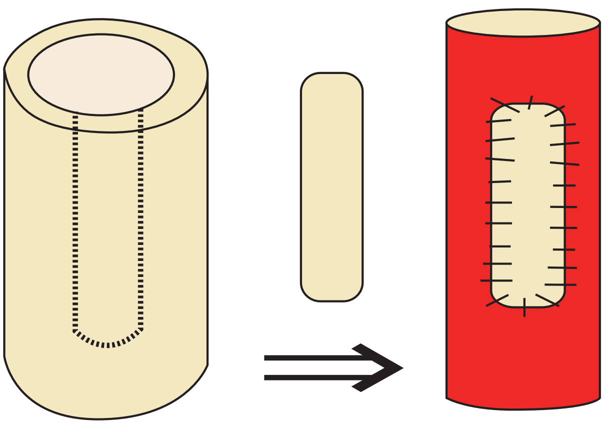


Fig. 25: Schematic illustration of shaping a Phe-based PEA tubular fibrous graft into a vascular patch to patch onto a defect of a rat carotid artery.

At the end of 14 days postimplantation, the patched carotid artery was explanted, processed and H&E stained as well as immunohistochemical stained for monoclonal rabbit antibody against macrophage for inflammation assessment, smooth muscle actin for intimal hyperplasia determination, and endothelial cells for endothelium lining analysis. Fig. 26 shows such histopathological data of the Phe-based PEA patch.

The data in Fig. 26 show that no intimal hyperplasia formation was found in the NOD-eluting Phe-based PEA patch site, and an endothelial lining on the top of the Phe-based PEA fibrous patch (left image) with a muted inflammatory response (right image) was also found at the 14 days post-implantation.

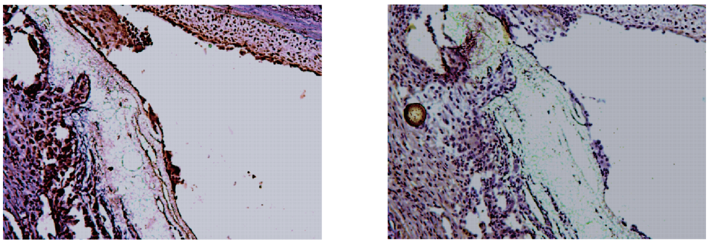


Fig. 26: Histopathological data of the NOD-eluting Phe-based PEA electrospun fibrous membrane patch of a rat carotid artery after 14 days. Left image is stained for endothelial cells, and Right image is stained for inflammatory cells. The endothelial cell lining (left) and the absence of inflammatory cells (right) are evident.

On the contrary, the PCL patch control showed no endothelium lining (Fig. 27, left) with severe inflammatory response (Fig. 27, right) and intimal hyperplasia formation. Some macrophages also infiltrated into the interior of the PCL fibrous membrane (indicated by a red arrow).

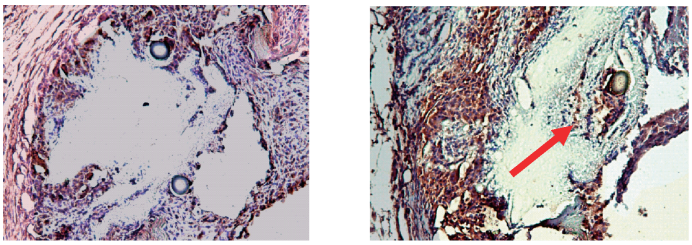


Fig. 27: Histopathological data of the absorbable poly-ε-caprolactine (PCL) fibrous patch of a rat carotid artery after 14 days. Left image was stained for endothelial cells, and the Right image was stained for macrophage for inflammation assessment. The infiltration of macrophages into the interior of the PCL fibrous patch is indicated by the red arrow.

This unpublished *in vivo* data in a rat carotid artery patch model clearly confirm other published *in vitro* and *in vivo* data of this new family of biodegradable amino acid-based poly(ester amide) biomaterials that they have the unique and favborable biological property: muted inflammatory response and support natural wound healing.

6.3 As Coating Biomaterial for Metallic Drug-eluting Stents – an *in vivo* Porcine Aortic Model Study [57]

Metallic stent devices are the standard choice of care for the percutaneous treatment of blocked coronary artery disease due to their proven acute [67] and long-term benefits [68, 69]. In-stent restenosis still remains a major problem, especially in unfavorable lesion subsets, such as diffuse-lesion, small-vessel or diabetic patients [70-72], even though subacute stent thrombosis has been reduced to less than 1% [73] with improved stent implantation techniques [74], antiplatelet therapy [75] and stent surface treatment and coating [76, 77]. As the mechanism of in-stent restenosis is predominantly neointimal hyperplasia [78], prevention will require concomitant antiproliferative and possibly antiinflammatory therapies. Thus, drug-coated stents containing an antiproliferative agent would be ideal to treat both the geometric remodeling [79] and neointimal hyperplasia components of restenosis. Indeed, clinical studies with rapamycin-coated stents [80] and paclitaxel-coated stents [81] have supported this approach with significant reduction in in-stent restenosis compared with bare metal stents. However, late stage thrombosis has been surfaced as the major complication from this generation of drug-eluting stents.

One requirement for successful drug-coated stent development is biocompatible and biologically inert polymers for drug impregnation to create a sustained drug release, as drugs can't be easily coated onto metallic stents by itself. The current commercial polymer coating materials used for both rapamycin-coated and Paclitaxel-coated stents are the non-biodegradable poly-n-butyl methacrylates (PBMA) of molecular weight 265,000 – 375,000.

Chu's lab developed a promising alternative to the current commercial drug-eluting stent technology in terms of the type of polymer coating and the type of drug. Instead of off-the-shelf non-biodegradable PBMA and poly(vinylidene fluoride)-Hexafluoropropylene copolymer coatings, the biodegradable functional amino acid-based poly(ester amide)s AA-PEA copolymer with conjugated nitric oxide derivative, NOD (4-amino-2.2.6.6-tetramethylpiperidine-1-oxy) as the drug based on the Chu's patent technology [11, 25, 82, 83]. An example of this NOD-tagged AA-PEA copolymer made of Leu and Lys amino acids is given below, where x and y are the number of methylene groups in the diacids and diol building blocks (refer to Fig. 1 for x and y).

$$\text{x-[Leu-y]}_{\text{m}} - \text{[Lys-NOD]}_{\text{n}}$$

Depending on the m to n ratio, the amounts of NOD conjugated onto the functional AA-PEA copolymers can be controlled. The m block is not limited to Leu, any other amino acid like Phe could also be used.

To evaluate the biocompatibility of this functional AA-PEA copolymer coating, Genic stents (316 stainless steel stent with expanded tubular slotted stents and 18% surface coverage) were coated by a particular 8-$[\text{Lue-6}]_{0.75}$–$[\text{Lys}]_{0.25}$ AA-PEA copolymer using a computer-controlled robotic dip-coating technology (Zynergy Core Technology, Runcorn, UK) with 1% weight per volume (w/v) AA-PEA solution in ethanol [57]. Fig. 19 shows such a AA-PEA coated Genic stent. This AA-PEA coated stent along with a bare stent as the control were deployed in Yorkshire swine (30–35 kg).

Both quantitative coronary angiography and histological data were obtained at the end of 4 weeks postimplantation and analyzed. This 4 week duration for in vivo biocompatibility is sufficient to distinguish biocompatibility from severe inflammatory reaction to the polymer as the majority of preclinical studies with polymer-coated stents [84-86] for evaluating the polymers had used such 1 month duration.

Fig. 28 shows the images of the porcine coronary arteries 28 days postimplantation. Table 1 summarizes the angiographic and histologic findings of both the AA-PEA coated and bare stents. The angiographic results indicated that all stents were widely patent at followup. There was only one animal with 450% diameter stenosis, but both the control and the polymer-coated stents exhibited a similar stenosis (51.5% for both). The angiographic data were confirmed by the histologic data which showed that there was normal appearance of media with mild compression at the stent wire sites. The neointima was thin and consists of smooth muscle cells with extracellular matrix. Only a few inflammatory cells were present adjacent to the stent strut. The neointimal and inflammatory responses are between the polymer-coated and the bare stents were comparable.

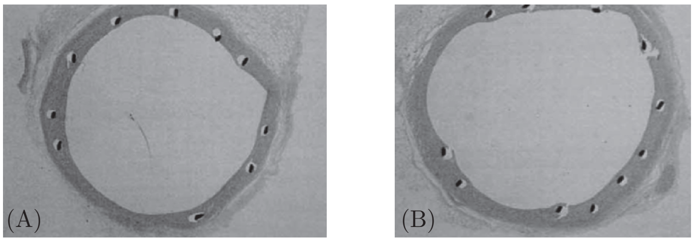


Fig. 28: Representative photomicrographs of porcine coronary arteries 28 days after AA-PEA polymer-coated stent (A) and bare stent (B). There is normal appearance of media with mild compression at the stent wire sites [57].

Table 1: Summary of the angiographic and histologic data [57]

Type	AA-PEA coated stents	Bare stents
Reference vessel (mm)	2.74 ± 0.4	2.74 ± 0.4
Stent/artery	1.18 ±0.15	1.18±0.15
Inflammatory score	1.18±0.38	1.11±0.32
Area stenosis (%)	26±17	28±22
Pervasculitis	1.21±0.37	1.13±0.28
Neointimal hyperplasia (mm^2)	1.46±0.71	1.69±1.16

A two years international multicenter human trial (45 patients) for assessing AA-PEA-NOD coated Genic coronary stents was reported by Costantini et al. [87]. No Major Adverse Cardiac Effect (MACE) was observed at 30 days, 12 and 24 months postimplantation, and no significant angiographic differences between 4 and 12 months based on quantitative coronary analysis. There was statistically significant lower ($p<0.01$) thickness of the neointimal hyperplasia in the 50% AA-PEA-NOD-coated stent vs. the bare stent. Based on the late loss and binary restenosis rate data as well as intravascular ultrasound volumetric analysis, no detrimental interaction between the AA-PEA-NOD and neointimal hyperplasia at a long-term follow up could be concluded.

In conclusion, both angiographic and histological findings showed similar inflammation and neointimal hyperplasia between the two groups. The results of this study suggest that the AA-PEA polymer is biocompatible and may be a suitable candidate as a stent coating. A human trial of these AA-PEA coated drug-eluting stent is currently in progress by a third party.

6.4 As Coating for Wound Closure Biomaterials - an *in vivo* Study

In addition as the coating for metallic drug-eluting stents described in 6.3 section above, AA-PEAs have also been studied as the coating materials for other medical devices like wound closure biomaterials and devices for the purpose of reducing inflammatory response of the coated devices.

Wound closure biomaterials and devices are required for every surgery and wound closure and they are made of both natural and synthetic, and absorbable and non-absorbable polymers and metals, [1, 2]. Among the nature-based wound closure biomaterials, catgut (reconstituted collagen) and silk are the most frequently used. Although these nature-based wound closure biomaterials have some good mechanical property like knot security and handling characteristics, they are foreign proteins to the human body, and hence are well-known for inducing far more intense tissue reaction than synthetic absorbable and most nonabsorbable wound closure biomaterials [1, 2].

Wound closure biomaterials and devices are frequently coated for improving their handling property like reducing tissue drags. The trend in using coating materials for wound closure biomaterials purpose is to develop coating materials that have a chemical property similar to the suture to be coated, and most of these coating materials are tailored toward synthetic absorbable and non-absorbable wound closure biomaterials. There is relatively little research and development of new coating materials for natural absorbable sutures like catgut and reconstituted collagen sutures.

Gaillard recently reported that the widely reported relatively poor *in vivo* biocompatibility and performance of collagen-based sutures could be improved by using biodegradable polymeric coating materials instead of the conventional chromium salts [88]. The rationale behind his approach is that a biodegradable protective coating could temporarily shield the substrate catgut sutures from enzymatic biodegradation during the early stage of wound healing so that the coated catgut sutures should be able to not only retain better strength in this critical period of wound healing but also delay and reduce the well-known adverse tissue reactions by delaying the onset of enzymatic biodegradation beyond the initial stage of wound healing. It is well-known that the rapid loss of tensile strength during the early stage of wound healing is one of the most important concerns for catgut sutures. By varying the composition and thickness of coating materials, Gaillard believed that a range of biodegradation properties of coated catguts could be achieved for a variety of clinical purposes. The use of synthetic biodegradable polymers as the coating for catgut sutures would also eliminate the known toxicity of chromium salts and improve knot tie-down and security. Gaillard's recipe for the biodegradable protective coating consists of biodegradable linear polyesters like glycolide or lactide reinforced with urea and urethane linkages.

In a preliminary trial, a simple Phe-based-PEA (8-Phe-4) was used to coat both 2/0 size plain catgut and silk sutures and implanted in the mouse gluteal muscles. The inflammatory zone surrounding the implanted sutures at the end of the 7 days postimplantation was measured as an indication of local tissue reaction. The corresponding uncoated sutures served as the control to assess the merit of the AA-PEA coating biomaterials.

Fig. 29 shows the measured inflammatory zone of both 8-Phe-4 coated and uncoated plain catgut sutures. The 8-Phe-4 coated plain catgut sutures had an inflammatory zone of only 33% of the uncoated plain catgut control at 7 days postimplantation. Similar significant reduction in the inflammatory zone was also found in the 8-Phe-4 coated silk suture. Therefore, this 7 days inflammatory zone data of the AA-PEA coated plain catgut and silk sutures clearly confirm all other *in vivo* and *in vitro* data that AA-PEA biomaterials indeed have muted inflammatory response, and would have a great potential as a coating material to reduce tissue inflammatory response of all existing medical devices of metallic or polymer nature.

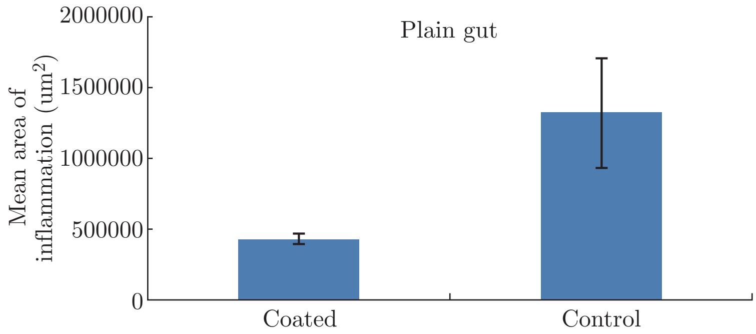


Fig. 29: Calculated inflammatory zone surrounding both the 8-Phe-4 coated and uncoated 2/0 size plain catgut sutures after 7 days in mouse gluteal muscle.

6.5 *In vitro* Gene Delivery Vehicle for Transfection Purpose

During the past several decades, with the fast growing molecular biology techniques, gene therapy technology has been developed rapidly. Since the first treatment of patients with gene transfer techniques under the approved FDA protocols in 1990, more than 1000 gene therapy clinical trials have been approved worldwide. However, the successful rate of gene therapy is not very encouraging. Based on the reported gene trial results, one of the key limitations is that there have not been safe, efficient and controllable methods for gene delivery [47, 89-96].

Gene delivery can be mainly accomplished by either virus or non-viral transfer methods. The advantages and disadvantages of viral delivery have been well documented [97]. Based on the aspect of the clinical safety, the non-viral gene delivery method appears to be the most promising approach.

For the reported non-viral gene delivery vectors, most of them can be divided into the following 4 broad categories: water soluble cationic polymers, lipids, dendrimers and nanoparticles. Among them, the water soluble synthetic and natural polycations have attracted the most attentions [94, 98-100]. A large number of cationic polymers have been tested for gene delivery. Among them, poly-L-lysine (PLL) [100-102] and polyethylenimine (PEI) [98, 103-105] have been intensively studied because of their strong interaction with the plasmid DNA, resulting the formation of a compact polymer/DNA complex. Other synthetic and natural polycations developed as non-viral vectors includes polyamidoamine dendrimers [106, 107] and chitosan [108-110], imidazole-containing polymers with proton-sponge effect [111-113], membrane-disruptive peptides and polymers like polyethylacrylic acid (PEAA) [114, 115], poly[alpha-(4-aminobutyl)-L-glycolic acid] (PAGA) [116], and poly(amino acid) based materials [117]. However, most of them could not achieve both high transfection efficiency and low toxicity simultaneously.

One of the new generations in the AA-PEA family developed very recently is the cationic Arg-based PEAs shown in Fig. 30 [24, 36, 39, 44, 48]. Most of these Arg-PEAs are water soluble, and due to the presence of the guanidino group which has an isoelectric point 10.96 and pKa 12.5, Arg-PEAs exhibit cationic charge over a wide range of pH including physiological pH 7.4. Hence, Arg-PEAs could be very good DNA condensation agents. In addition, Arg-based polymers have been shown to enter cells efficiently, suggesting that Arg-PEA/DNA complex could enter cells easily for transfection purpose.

The use of Arg-based PEAs for gene transfection purpose has been extensively studied in the Chu's lab [39, 48]. A wide range of cell lines, primary cells and stem cells like rat A10 vascular

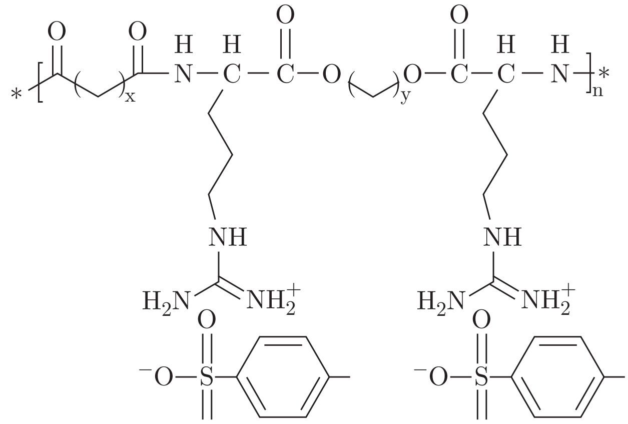


Fig. 30: Generic chemical structure of the repeating unit of Arg-PEA

smooth muscle cells, human umbilical vein endothelial cells, macrophages, rat aortic fibroblasts, and mesenchymal stem cells, lymphoma cells, was investigated for the transfection capability as well cytotoxicity of these new generations of water soluble cationic Arg-based PEAs. Fig. 31 shows some representative transfection data of rat A10 smooth muscle cell line based on fire fly luciferase assay [39].

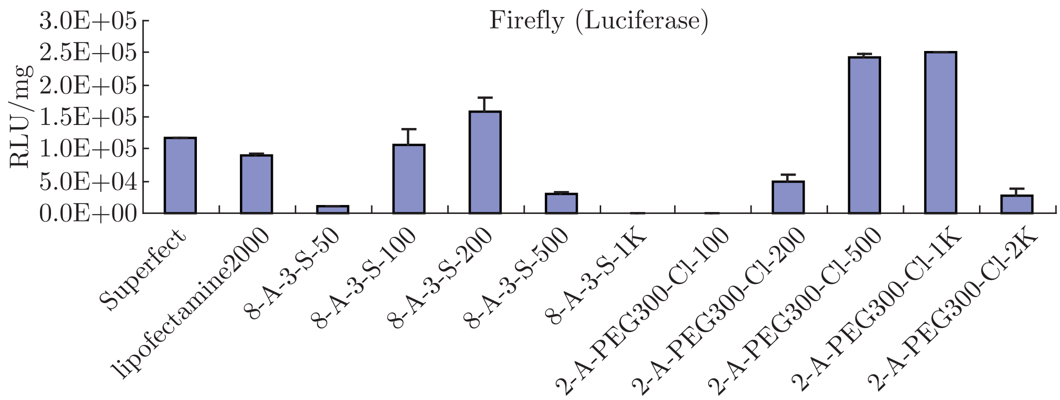


Fig. 31: *In vitro* transfection data (based on firefly luciferase) of 2 different generations of the Arg-based PEAs at different weight ratio of the polymer to DNA. The transfection time was 4 hrs. Both commercial Lipofectamine 2000® and Superfect® transfection agents were used as the control (the 2 on the far left).

The transfection data in Fig. 31 clearly demonstrate that some of these Arg-PEAs (e.g., 2-Arg-PEG300-Cl-500, and 8-Arg-3-S-200 exhibit far better transfection efficiency than both commercial controls. The most unique property of these Arg-based PEAs as gene transfection agents is that they can achieved better transfection than commercial transfection agents (Lipofectaine2000® and Superfect®) and the most popular experimental transfection agent (PEI) at a far lower cytotoxicity as shown in Fig. 32.

The quantitative MTT data illustrate the outstanding cell biocompatibility of these new Arg-PEA transfection agents, and these quantitative MTTdata were further confirmed by the qualitative rat aortic smooth muscle cell A10 cell morphology data as shown in Fig. 16.

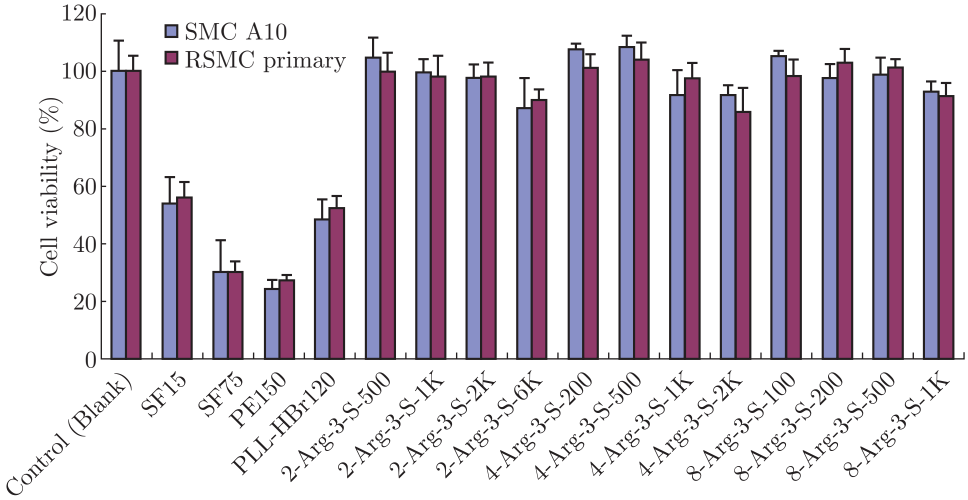


Fig. 32: Cytotoxicity of a series of Arg-PEA/DNA complexes after 48 hrs incubation with rat aortic smooth muscle cell A10 cell line (SMC A10) and primary cells (RSMC Primary). Blank and commercial Superfect® (SF), experimental PEI and poly-L-lysine (PLL) were used as the controls. The numeric # after each transfection agent label is the weight ratio of that agent to DNA.

7 Conclusion

A new family of synthetic biodegradable amino acid-based poly(ester amide)s (AA-PEAs) has very recently been designed and developed for the purpose of providing a new class of biomaterials for significantly improved performance of medical devices. These AA-PEAs are designed from 3 building blocks: amino acids, fatty diols and fatty diacids. Due to the enormous variety of these 3 building blocks, a wide range of AA-PEAs could be tailor-designed for meeting specific clinical needs. Many generations of AA-PEAs have been developed, depending on the type of the 3 building blocks. For example, the use of ionic/polar amino acids like Arg or Lys, cationic and anionic AA-PEAs have been designed, while the use of non-polar amino acids like Phe or Leu, hydrophobic and neutral charged AA-PEAs have been designed. These AA-PEAs have been engineered into a variety of physical forms ranging from 3D microporous hydrogels, melt-spun fibers, electrospun fibrous membranes, films and microspheres. All these AA-PEAs have shown 2 unique biological properties: support natural wound healing, and muted inflammatory response. When AA-PEAs are chemically coupled with other biomaterials, the muted inflammatory response characteristics of AA-PEAs can also be extended to the coupled biomaterials. Many animal and few human trials have established the safety aspect of this new family of synthetic biodegradable biomaterials. AA-PEAs have been explored for a variety of biomedical applications ranging from treating burns, as coating for drug-eluting stents, drug delivery vehicles, gene transfection agents, scaffolds for tissue engineering and synthetic vaccines.

Acknowledgement

The work and data cited in this review are largely from my past and present collaborators, graduate students and postdoctors. With their efforts, the bulk of the data have become available for me to compile this review.

References

[1] Chu CC, von Fraunhofer JA, and Greisler HP. Wound Closure Biomaterials and Devices. Boca Raton, Fla: CRC Press, 1997.

[2] Chu CC, An overview of surgical suture biomaterials. In: King M, and Gupta BS, editors. Biotextiles as Medical Implants. Cambridge, England: Woodhead Publishing, In Press.

[3] Pillai CKS and Chandra P. Sharma. Absorbable polymeric surgical sutures: Chemistry production, properties, biodegradability, and performance. J Biomater Appl 2010; 25: 291-366.

[4] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric Systems for Controlled Drug Release. Chemical Reviews (Washington, D C) 1999; 99(11): 3181-3198.

[5] Langer R. New methods of drug delivery. Science (New York, NY) 1990; 249(4976): 1527-1533.

[6] Barrera DA, Zylstra E, Lansbury PT, Jr., Langer R. Synthesis and RGD peptide modification of a new biodegradable copolymer: poly(lactic acid-co-lysine). J of the Am Chem Soc 1993; 115(23): 11010-11011.

[7] Barrera DA, Zylstra E, Lansbury PT, Langer R. Copolymerization and degradation of poly(lactic acid-co-lysine). Macromolecules 1995; 28(2): 425-432.

[8] Langer R, Tirrell DA. Designing materials for biology and medicine. Nature (London, United Kingdom) 2004; 428(6982): 487-492.

[9] Wang Y, Ameer GA, Sheppard BJ, Langer R. A tough biodegradable elastomer. Nature Biotechn 2002; 20(6): 602-606.

[10] West JL. Drug delivery: Pulsed polymers. Nature Materials 2003; 2(11): 709-710.

[11] Chu CC, and Katsarava R. Elastomeric functional biodegradable copolyester amides and copolyester urethanes, US Patent No. 6,503,538, 2003.

[12] Katsarava R, Beridze V, Arabuli N, Kharadze D, Chu CC, and Won CY. Amino acid based bioanalogous polymers. Synthesis and study of regular poly (ester amide)s based on bis(a-amino acid) α, ω-alkylene diesters and aliphatic dicarboxylic acids. J. Polym. Sci., Polymer Chemistry Ed. 1999; 37: 391-407.

[13] Paredes, N, Casas MT. Puiggali J. Packing of sequential poly(ester amide)s derived from diols, dicarboxylic acids, and amino acids. Macromolecules 2000; 33: 9090-9097.

[14] Barrows, TH. Degradable implant materials: A review of synthetic absorbable polymers and their applications. Clin. Mater. 1986; 1: 233-257.

[15] Bettinger CJ, Bruggeman JP, Borenstein JT, Langer RS. Amino alcohol-based degradable poly(ester amide) elastomers. Biomaterials 2008; 29: 2315-2325.

[16] Garg P, Keul H, Klee D, Moeller M. Thermal properties of poly(ester amide)s with isolated, two adjacent and three adjacent amide groups within a polyester chain. Macromol. Chem. Phys. 2009; 210: 1754-1765.

[17] Kim, TH, Cook SE, Arote RB, Cho MH, Nah J W, Choi YJ, Cho CS. A degradable hyperbranched poly(ester amine) based on poloxamer diacrylate and polyethylenimine as a gene carrier. Macromol. Biosci. 2007; 7: 611-61.

[18] 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: 1271-1282.

[19] Asin L, Armelin E, Montane J, Rodriguez-Galan A, Puiggali 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: 4283-4293.

[20] 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: 3453-3462.

[21] Guo K, Chu CC. Copolymers of unsaturated and saturated poly(ether ester amide)s: Synthesis, characterization, and biodegradation. J. Appl. Polym. Sci. 2008; 110: 1858-1869.

[22] 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.

[23] Chkhaidze E, Tugushi D, Kharadze D, Gomurashvili Z, Chu CC, Katsarava R. New unsaturated biodegradable poly(ester amide)s composed of fumaric acid, L-leucine and α, ω-alkylene diols. J. Macromol. Sci. Part A 2011: 48: 544-555.

[24] Wu, J, Mutschler MA, Chu CC. Synthesis and characterization of ionic charged water soluble arginine-based poly (ester amide). J. Mater. Sci. Mater in Med. 2011: 22: 469-479.

[25] Jokhadze G, Machaidze M, Panosyan H, Chu CC, Katsarava R. Synthesis and characterization of functional elastomeric poly(ester amide) co-polymers. J. Biomater. Sci. Polymer Edn. 2007: 18: 411-438.

[26] 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: 2851-2861.

[27] Tsitlanadze G, Machaidze M, Kviria T, Djavakhishvili N, Chu CC, Katsarava R. Biodegradation of amino-acid-based poly(ester amide)s: *In vitro* weight loss and preliminary *in vivo* Studies. J. Biomater. Sci. Polym. Ed. 2004; 15: 1-24.

[28] Li L, Chu CC. Nitroxyl radical incorporated electrospun biodegradable poly(ester amide) nanofiber membranes J. Biomater. Sci. Polym. Ed. 2009; 20: 341-361.

[29] Guo K, Chu CC. Biodegradation of unsaturated poly(ester amide)s and their hydrogels. Biomaterials 2007; 28: 3284-3294.

[30] Katsarava R. Active polycondensation: From peptide chemistry to amino acid based biodegradable polymers. Macromol. Symp. 2003; 199: 419-429.

[31] Fan YJ, Kobayashi M, Kise H. Synthesis and specific biodegradation of novel polyester amides containing amino acid residues. J. Polym. Sci., Part A: Polym. Chem. 2001; 39: 1318-1328.

[32] Horwitz JA, Shum KM, Bodle JC, Deng MX, Chu CC, Reinhart-King CA. Biological performance of biodegradable amino acid-based poly(ester amide)s: Endothelial cell adhesion and inflammation *in vitro*. J. Biomed. Mater. Res. Part A, 2010; 95: 371-380.

[33] Deng MX, 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 Biomaterialia 2011; 7: 1504-1515.

[34] 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: 3037-3047.

[35] Pang X, Chu CC. Synthesis, characterization and biodegradation of novel poly(ester amide)s and their functionalization. Biomaterials 2010; 31: 3745-3754.

[36] Pang X, Wu J, Reinhart-King C, and Chu CC. Synthesis and characterization of functionalized water soluble cationic poly(ester amide)s. J. Polym. Sci. Part A: Polym. Chem. 2010; 48: 3758-3766.

[37] De Wit MA, Wang ZX, 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: 6376-6392.

[38] 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: 1495-1511.

[39] Wu J. Arginine and Phenylalanine Based Poly(ester amide)s: Synthesis, Characterization and Applications. PhD thesis, Cornell University, Aug. 5, 2010.

[40] Schwartz S, Demars S, Chu CC, White J, Cooper A, Rothrock M, Adelman M, Yurt R. Efficacy of poly(ester amide) dressings on partial thickness wound healing. 3rd World Union of Wound Healing Societies. 2008, June 4-8, in Toronto, Canada.

[41] Vera M, Almontassir A, Rodriguez-Galan, A.; Puiggali, J. Synthesis and characterization of a new degradable poly(ester amide) derived from 6-amino-1-hexanol and glutaric acid. Macromolecules 2003; 36: 9784-9796.

[42] Botines E, Casas MT, Puiggali J. Alternating poly(ester amide)s of glycolic acid and ω-amino acids: Crystalline morphology and main crystallographic data. J. Polym. Sci. Part B: Polym. Phys. 2007; 45: 815-825.

[43] Armelin E, Paracuellos N, Rodriguez-Galan A, Puiggali J. Study on the degradability of poly(ester amide)s derived from the α-amino acids glycine, and L-alanine containing a variable amide/ester ratio. Polymer, 2001; 42: 7923-7932.

[44] Song H, Chu CC. Synthesis and characterization of a new family of cationic poly(ester amide)s and their biological properties. J. Appl. Polym. Sci. 2011. (In Press).

[45] Rodriguez-Galan A, Pelfort M, Aceituno JE, Puiggali J. Comparative studies on the degradability of poly(ester amide)s derived from L- and L,D-alanine. J. Appl. Polym. Sci. 1999; 74: 2312-2320.

[46] Guo K, Chu CC. Synthesis of biodegradable amino acid-based poly(ester amide) and poly(ether ester amide) with pendant functional groups. J. Appl. Polym. Sci. 2010; 117: 3386-3394.

[47] Yamanouchi D, Wu J, Lazar AN, Craig Kent K, Chu CC, Liu B. Biodegradable arginine-based poly(ester-amide)s as non-viral gene delivery reagents. Biomaterials 2008; 29(22): 3269-3277.

[48] Song H. L-arginine-based biodegradable poly(ester amide)s, their synthesis, characterization, fabrication, and application as drug and gene carriers. 2007, PhD thesis, Cornell University: Ithaca, NY, USA.

[49] Chu C, and Sun D. New electrospun synthetic biodegradable poly(ester amide) drug-eluting fibrous membranes for potential wound treatment. in Medical, Nonwovens, and Technical Textiles. 2008. Durham NC.

[50] Guo K, and Chu CC. Biodegradable and injectable paclitaxel-loaded poly(ester amide)s microspheres: Fabrication and characterization. J Biomed Mater Res Part B-Applied Biomaterials, 2009; 89B(2): 491-500.

[51] Pang X, and Chu C. Synthesis, characterization and biodegradation of poly(ester aminde)s based hydrogels. Polymer, 2010; 51: 4200-4210.

[52] Guo K, and Chu CC. Controlled release of Paclitaxel from biodegradable unsaturated poly(ester amide)s / poly(ethynene glycol) diacrylate hydrogels. J Biomater Sci Polym Ed., 2007; 18(5): 489-504.

[53] Guo K, Chu CC, Chkhaidze E, and Katsarava R. Synthesis and characterization of novel biodegradable unsaturated poly(ester-amide)s, J Polym Sci Polym Chem, 2005; 43: 1463-1477.

[54] Lewis P, and Chu C. Novel fibrous scaffold for wound management, In: The Fiber Society 2004 Fall Symposium on Advanced Materials and Processes, Oct. 11-13, 2004, Cornell University, Ithaca, NY.

[55] Anderson JM, Rodriguez A, and Chang DT. Foreign body reactions to biomaterials, Seminars in Immunology, 2008; 20: 86-100.

[56] Whitaker MJ, Wuirk RA, Howdie SM, Shakesheff KM. Growth factor release from tissue engineering scaffolds. J. Pharm. Pharmacol, 2001; 53(11): 1427-1437.

[57] Lee SH, Szinai I, Carpenter K, Katsarava R, Jokhadze G, Chu CC, De Scheerder I, and Hong MK, *In vivo* biocompatibility evaluation of stents coated by a new biodegradable elastomeric and functional polymer. Coronary Artery Disease, 2002; 13(4): 237-241.

[58] http://www.ameriburn.org/resources_factsheet.

[59] Petrie NC, Yao F, Eriksson E. Gene therapy in wound healing. Surg Clin N Am 2003; 83(3): 194-199.

[60] Advanced Medical Technology Association. Advanced Wound Management: Healing and Restoring Lives. June 2006. www.advamed.org/NR/rdonlyres/8D8348E8-811D-48A9-8737 61D91060DDAD/ 0/june2006_woundmanagement.pdf.

[61] Samsom D, Lefevre F, Aronson N. Wound Healing Technologies: Low-Level Laser and Vacuum-Assisted Closure. Summary, Evidence Report/Technology Assessment No. 111. (Prepared by the Blue Cross and Blue Shield Association Technology Evaluation Center Evidence-based Practice Cener, under Contract No. 290- 02-0026). AHRQ Publication No. 05-E005-1. Rockville, MD: Agency for Healthcare Research and Quality. December2004.

[62] Atiyeh BS, Hayek SN, Gunn SW. New technologies for burn wound closure and healing–Review of the literature. Burns 2005; 31: 944-956.

[63] The Joanna Briggs Institute. Split Skin Graft Donor Sites: Post Harvest Management. Best Practice 2002; 6(2): 1-6.

[64] Ehrenreich M, Ruszczak Z. Update on tissue-engineered biological dressings. Tissue Engineering 2006; 12(9): 2407-24.

[65] Zilla P and Greisler HP. Tissue engineering of vascular prosthetic grafts. Austin, TX, R.G. Landes, Co., 1999.

[66] Chu CC and Liu B. Novel biodegradable scaffolds for tissue engineering of blood vessels, Annual Report to the Morgan Tissue Engineering Program, Jan. 28, 2008, Cornell University, Ithaca, New York.

[67] Lindsay J Jr, Hong MK, Pinnow E, Pichard AD. Effects of intraluminal coronary stents on the frequency of coronary artery bypass grafting after unsuccessful percutaneous transluminal coronary revascularization. Am J Cardiol 1996; 77: 647-649.

[68] Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, et al. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. N Engl J Med 1994; 331: 496-501.

[69] Serruys PW, de Jaegere P, Kiemeneij F, Macaya C, Rutsch W, Heyndrickx G, et al. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. N Engl J Med 1994; 331: 489-495.

[70] Kobayashi Y, De Gregorio J, Kobayashi N, Akiyama T, Reimers B, Finci L, et al. Stented segment length as an independent predictor of restenosis. J Am Coll Cardiol 1999; 34: 651-659.

[71] Akiyama T, Moussa I, Reimers B, Ferraro M, Kobayashi Y, Blengino S, et al. Angiographic and clinical outcome following coronary stenting of small vessels. A comparison with coronary stenting of large vessels. J Am Coll Cardiol 1998; 32: 1610-1618.

[72] Abizaid A, Kornowski R, Mintz GS, Hong MK, Abizaid AS, Mehran R, et al. The influence of diabetes mellitus on acute and late clinical outcomes following coronary stent implantation. J Am Coll Cardiol 1998; 32: 584-589.

[73] Cutlip DE, Baim DS, Ho KK, Popma JJ, Lansky AJ, Cohen DJ, et al. Stent thrombosis in the modern era: a pooled analysis of multicenter coronary stent clinical trials. Circulation 2001; 103: 1967-1971.

[74] Colombo A, Hall P, Nakamura S, Almagor Y, Maiello L, Martini G, et al. Intracoronary stenting without anticoagulation accomplished with intravascular ultrasound guidance. Circulation 1995; 91: 1676-1688.

[75] Leon MB, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, et al. A clinical trial comparing three antithrombotic-drug regimens after coronary artery stenting. N Engl J Med 1998; 339: 1665-1671.

[76] Hardhammar PA, van Beusekom HM, Emanuelsson HU, Hofma SH, Albertsson PA, Verdouw PD, et al. Reduction in thrombotic events with heparin-coated Palmaz–Schatz stents in normal porcine coronary arteries. Circulation 1996; 93: 423-430.

[77] Serruys PW, Emanuelsson H, van der Giessen W, Lunn AC, Kiemeney F, Macaya C, et al. Heparin-coated Palmaz–Schatz stents in human coronary arteries: early outcome of the BENESTENT-II pilot study. Circulation 1996; 93: 412-422.

[78] Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1992; 20: 467-474.

[79] Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong SC, et al. Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study. Circulation 1996; 94: 35-43.

[80] Fajadet J, Perin M, Hayashi EB, Colombo A, Schuler G, Barragan P, et al. 210-day follow-up of the RAVEL study: a randomized study with the sirolimus-eluting BX VELOCITY balloon expandable stent in the treatment of patients with de novo native coronary artery lesions [abstract]. J Am Coll Cardiol 2002; 39(suppl A): 20A.

[81] Park SJ, Shim WH, Ho DS, Raizner A, Park SW, Kim JJ, et al. The clinical effectiveness of paclitaxel-coated coronary stents for the reduction of restenosis in the ASPECT trial [abstract]. Circulation 2001; 104(Suppl II): II-464.

[82] Chu CC, Lee KH, and Freed J. Aminoxyl-containing radical spin labeling in polymers and copolymers, U.S. Patent No. 5, 516, 881, 1996.

[83] Lee KH & Chu CC. Molecular design of biologically active biodegradable polymers for biomedical applications, Macromol. Symposium (Huthig & Wepf Verlag, Zug) 1998; 130: 71-80.

[84] van der Giessen WJ, Lincoff AM, Schwartz RS, van Beusekom HMM, Serruys PW, Holmes DR Jr, et al. Marked inflammatory sequelae to implantation of biodegradable and non-biodegradable polymers in porcine coronary arteries. Circulation 1996; 94: 1690-1697.

[85] Lincoff AM, Furst JG, Ellis SG, Tuch RJ, Topol EJ. Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. J Am Coll Cardiol 1997; 29: 808-816.

[86] Hong MK, Kornowski R, Bramwell O, Ragheb AO, Leon MB. Paclitaxel-coated Gianturco–Roubins stents reduce neointimal hyperplasia in a porcine coronary in-stent restenosis model. Coron Artery Dis 2001; 12: 513-515.

[87] Costantini CR, Londero H, DeScheeder I, Tarbine SG, Costantini CO, Cabrera MJ, Santos MF, Darwich RZ, Maranhao MC, Bubna M, Andrade MS, Yared A. "Final results and two year outcomes of a trial assessing a nitric oxide preserver polymer-coated stent implantation in De Novo Coronary lesions: Clinical, angiographic, and stent volumetric analysis from NOBLESSE trial. Am. J. Cardiol. 2005; 96 (suppl 7A): 39H.

[88] Gaillard BD. Sheathed surgical suture filament and method for its preparation, U.S. Patent No. 4, 506, 672, 1985.

[89] Mulligan RC. The Basic Science of Gene-Therapy. Science (New York, N.Y.) 1993; 260 (5110): 926-932.

[90] Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. Nature Reviews Drug Discovery 2005; 4 (7): 581-593.

[91] Wolff JA, Lederberg J., An early history of gene-transfer and therapy. Human Gene Therapy 1994; 5(4): 469-480.

[92] Wolff JA, Trubetskoy VS. The Cambrian period of nonviral gene delivery. Nature Biotechnology 1998; 16(5): 421-422.

[93] Lynn DM, Anderson DG, Putnam D, Langer R. Accelerated discovery of synthetic transfection vectors: Parallel synthesis and screening of degradable polymer library. J of the Am Chem Soc 2001; 123(33): 8155-8156.

[94] Mahato RI, Smith LC, Rolland A. Pharmaceutical perspectives of nonviral gene therapy. In: Hall J, Dunlap J, Friedmann, T, Giannelli F, editors. Advances in Genetics, New York: Academic Press, 1999. Vol. 41, pp 95-156.

[95] Putnam D, Polymers for gene delivery across length scales. Nature Materials 2006; 5(6): 439-451.

[96] Putnam D, Gentry CA, Pack DW, Langer R. Polymer-based gene delivery with low cytotoxicity by a unique balance of side-chain termini. Proceedings of the National Academy of Sciences of the United States of America 2001, 98, (3), 1200-+.

[97] Luo D, Saltzman WM. Synthetic DNA delivery systems. Nature biotechnology 2000: 18(1): 33-37.

[98] Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine. Proc Natl Acad Sci U S A 1995; 92(16): 7297-301.

[99] Verma IM, Somia N. Gene therapy - promises, problems and prospects. Nature 1997; 389 (6648): 239-242.

[100] Zauner W, Ogris M, Wagner E. Polylysine-based transfection systems utilizing receptor-mediated delivery. Advanced Drug Delivery Reviews 1998; 30(1-3): 97-113.

[101] Katayose S, Kataoka K. Water-Soluble Polyion Complex Associates of DNA and Poly(ethylene glycol)-Poly(L-lysine) Block Copolymer. Bioconjugate Chemistry 1997; 8(5): 702-707.

[102] Wagner E, Zatloukal K, Cotten M, Kirlappos H, Mechtler K, Curiel DT, Birnstiel ML. Coupling of adenovirus to transferrin-polylysine/DNA complexes greatly enhances receptor-mediated gene delivery and expression of transfected genes. Proceedings of the National Academy of Sciences of the United States of America 1992; 89(13): 6099-103.

[103] Zhong Z, Feijen J, Lok MC, Hennink WE, Christensen LV, Yockman JW, Kim YH, Kim SW. Low molecular weight linear polyethylenimine-b-poly(ethylene glycol)-*b*-polyethylenimine triblock copolymers: synthesis, characterization, and in vitro gene transfer properties. Biomacromolecules 2005; 6(6): 3440-3448.

[104] Pollard H, Remy JS, Loussouarn G, Demolombe S, Behr JP, Escande D. Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus in mammalian cells. J of Biol Chem 1998; 273(13): 7507-7511.

[105] Liu X, Yang JW, Lynn DM. Addition of "charge-shifting" side chains to linear poly(ethyleneimine) enhances cell transfection efficiency. Biomacromolecules 2008; 9(7): 2063-2071.

[106] Tekade RK, Kumar PV, Jain NK. Dendrimers in Oncology: An expanding horizon. Chemical Reviews (Washington, DC, United States) 2009; 109(1): 49-87.

[107] Tomalia DA, Naylor AM, Goddard WA. Starburst Dendrimers - Molecular-Level Control of Size, Shape, Surface-Chemistry, Topology, and Flexibility from Atoms to Macroscopic Matter. Angewandte Chemie-International Edition in English 1990; 29(2): 138-175.

[108] Wong K, Sun G, Zhang X, Dai H, Liu Y, He C, Leong KW. PEI-g-chitosan, a novel gene delivery system with transfection efficiency comparable to polyethylenimine *in vitro* and after liver administration *in vivo*. Bioconjugate Chemistry 2006; 17(1): 152-158.

[109] Koeping-Hoeggard M, Varum KM, Issa M, Danielsen S, Christensen BE, Stokke BT, Artursson P. Improved chitosan-mediated gene delivery based on easily dissociated chitosan polyplexes of highly defined chitosan oligomers. Gene Therapy 2004; 11(19): 1441-1452.

[110] Wu J, Wang X, Keum JK, Zhou H, Gelfer M, Avila-Orta CA, Pan H, Chen W, Chiao SM, Hsiao BS, Chu B. Water soluble complexes of chitosan-g-MPEG and hyaluronic acid. J of Biomed Mater Res, Part A 2007; 80A(4): 800-812.

[111] Ihm JE, Han KO, Han IK, Ahn KD, Han DK, Cho CS. High transfection efficiency of poly(4-vinylimidazole) as a new gene carrier. Bioconjugate Chemistry 2003; 14(4): 707-708.

[112] Midoux P, Monsigny M. Efficient gene transfer by histidylated polylysine pDNA complexes. Bioconjugate Chemistry 1999; 10(3): 406-411.

[113] Pack DW, Putnam D, Langer R. Design of imidazole-containing endosomolytic biopolymers for gene delivery. Biotechnology and Bioengineering 2000; 67(2): 217-223.

[114] Thomas JL, Tirrell DA. Polyelectrolyte-sensitized phospholipid-vesicles. Accounts of Chemical Research 1992; 25(8): 336-342.

[115] Thomas JL, Barton SW, Tirrell DA. Membrane solubilization by a hydrophobic polyelectrolyte - surface-activity and membrane-binding. Biophysical J 1994; 67(3): 1101-1106.

[116] Lim YB, Kim CH, Kim K, Kim SW, Park JS. Development of a safe gene delivery system using biodegradable polymer, poly[alpha-(4-aminobutyl)-L-glycolic acid]. J of the Am Chem Soc 2000; 122(27): 6524-6525.

[117] Lynn DM, Langer R. Degradable poly(beta-amino esters): Synthesis, characterization, and self-assembly with plasmid DNA. J Am Chem Soc 2000; 122(44): 10761-10768.