Constituents and Morphologies of Polymeric Nanocarriers

Subjects: Nanoscience & Nanotechnology

Contributor: Natalia Łopuszyńska , Władysław P. Węglarz

Typically, solid colloidal particles with a diameter of less than 1000 nm are referred to as nanocarriers. However, to avoid rapid clearance after intravenous administration, prolong the circulation half-life, and at the same time increase the likelihood of crossing various biologic barriers while preventing accumulation in capillaries and healthy tissues, the most common nanoparticle size for drug delivery referred to in the literature is between 100 and 500 nm. Depending on their internal structure, polymeric nanocarriers may be further classified as nanospheres (nanoparticles) or nanocapsules (NCs).

MRI theranostics drug delivery

1. The Nanocarriers Morphologies

Typically, solid colloidal particles with a diameter of less than 1000 nm are referred to as nanocarriers. However, to avoid rapid clearance after intravenous administration, prolong the circulation half-life, and at the same time increase the likelihood of crossing various biologic barriers while preventing accumulation in capillaries and healthy tissues, the most common nanoparticle size for drug delivery referred to in the literature is between 100 and 500 nm [1][2][3][4]. Depending on their internal structure, polymeric nanocarriers may be further classified as nanospheres (nanoparticles) or nanocapsules (NCs). Nanospheres are generally homogenous matrix systems in which the drug is dispersed in the material forming them. The drug can be adsorbed in their pores or less frequently at their surface, or it can be conjugated to them. Nanocapsules, on the other hand, are made up of two parts: the core and the shell. The core material can be solid, liquid, or gaseous, depending on the type of application, while the shell is formed by polymeric materials. In most cases, the drug is located in the core of the nanocapsule, while the shell protects it from the outside environment. Nevertheless, formulations with drugs incorporated in the shell or adsorbed at their surface are also possible, allowing the simultaneous delivery of one or several drugs in different nanocapsule compartments. The shell may be made permeable, semi-permeable, or impermeable, depending on the application, like the controlled release applications [1][5].

Due to their core–shell microstructure, polymeric nanocapsules have gained increased attention in recent years for use in drug delivery applications. The solid/oil core of nanocapsules can significantly improve drug loading efficiency while lowering the amount of polymeric matrix in nanoparticles when compared to polymeric nanospheres. Additionally, the polymeric shell can separate the encapsulated payload from the tissue environment, preventing the degradation or burst release caused by pH, temperature, enzymes, and other variables.

Furthermore, the polymeric shell can be functionalized by smart molecules capable of interacting with specific biomolecules, allowing for targeted drug delivery [1][6][7][8].

Three morphologies based on drug incorporation mechanisms are most commonly investigated for drug delivery. First are polymeric carriers that use covalent bonds for direct drug conjugation, including linear polymers, hyperbranched polymers, and dendrimers. Dendrimers are one of the major classes of polymers. They are synthesized with a central core and monomers that branch out radially from this core in a way that resembles a tree [9][10]. The second group of nanocarriers is based on hydrophobic interactions between the cargo and nanocarriers and includes polymeric micelles from amphiphilic block copolymers [11][12]. The third group includes polymersomes, which are structures obtained by the self-assembly of amphiphilic block copolymers. As a result of their inner hydrophilic compartment, these nanostructures are more suitable for the delivery of water-soluble agents [13].

Nanoprecipitation, emulsion–diffusion, double emulsification, emulsion–coacervation, polymer-coating, and layer-by-layer (LbL) are the six classical procedures for the preparation of nanocarriers. Nonetheless, additional approaches, such as emulsion–evaporation and polymer liposome production procedures, have been employed [14] as well.

2. Polymers for Nanocarrier Preparation

Polymer characteristics have a large impact on the stability, encapsulation efficiency, release profile, and biodistribution of the nanocapsule as a drug delivery vehicle. Biocompatible polymeric materials have been intensively investigated as potential compounds for the production of nanocapsules. In most cases, these polymers should be biodegradable in order to achieve the goals of payload release and nanoparticle elimination. Non-biodegradable yet biocompatible polymers such as polyethylene glycol (PEG) and polyvinyl alcohol (PVA) have also been frequently employed in the fabrication of nanoparticles. Because of their hydrophilicity, they can assist in drug release via diffusion. Furthermore, while not being degraded into smaller molecules, they might eventually be eliminated from circulation via the reticuloendothelial system [15][16]. To meet the diverse application requirements, several polymers have been used in the formulation of nanocapsules. These may be classified as natural or synthetic polymers based on their origin [5].

Peptides, proteins, nucleic acids, dextran ester, and chitosan are examples of natural polymers used. Because of their interactions with drug molecules, these molecules benefit from excellent biocompatibility but have short half-lives, non-specific distribution rates, and limited applications. Consequently, synthetic polymeric nanoparticles were proposed. The most important molecules exploited for such platforms are polylactic acid (PLA), poly(ε-caprolactone) (PCL), poly(lactide-co-glycolide) (PLGA), and poly(alkyl cyanoacrylate). Nevertheless, various other synthetic polymeric nanocarriers have also been reported, including polyaspartamide (PA), poly(L-aspartate), poly(D,L-lactic acid-co-glycolic acid), poly(ethylene glycol) (PEG), poly(N-vinyl pyrrolidone) (PVP), poly(N-isopropyl acrylamide) (PNIPAM), poly(hydroxypropyl methacrylamide) (PHPMA), poly(methyl methacrylate), poly-

(chloromethyl-styrene) (PCMS), etc. [17]. Polymers that were the most frequently used for the synthesis of MRI-detectable drug delivery systems are briefly described below.

2.1. Poly(ε-caprolactone) (PCL)

PCL is a semi-crystalline polymer that is insoluble in alcohol and water and soluble in non-polar solvents like benzene, chloroform, and carbon tetrachloride. It is slightly soluble in acetone, DMF, ethyl acetate, and acetonitrile. Its insolubility in polar solvents is one of the major issues with the application of PCL and the synthesis of PCL-based nanocarriers [18][19].

On the other hand, this hydrophobic nature promotes efficient cellular uptake [20]. PCL has proven biodegradability, biocompatibility, and FDA approval for human use [21][22]. In early in vivo studies, it was observed that PCL undergoes an initial hydrolytic degradation process via ester cleavage until its molar mass is sufficiently low to allow further intracellular degradation [23][24]. The PCL degradation process involves its ultimate conversion to 6-hydroxycaproic acid, which is completely metabolized in the human body [25], which is essential for easy removal from the body after its application and makes PCL a perfect candidate for the design of drug delivery systems. Degradation of PCL is monitored by changes in molecular weight and can be tailored by its synthesis method from months to years by impacting factors like the degree of crystallization, molecular weight, and morphology [21][26]. Moreover, it has been found that PCL is excreted through urine and feces [25].

PCL is characterized by a melting temperature significantly above body temperature, ranging between 59 and 64 °C, and a glass transition temperature of −60 °C, and thus it maintains its semi-crystalline state in physiological temperature conditions [27].

Two main strategies are used for the synthesis of this polymer: polycondensation and ring-opening polymerization. Nevertheless, the green synthesis strategy has been gaining popularity among researchers to overcome toxicity issues and safety concerns [28].

2.2. Polyethylene Glycol (PEG)

PEG is a polyether consisting of ethoxy units derived from the ring-opening polymerization of ethylene oxide. The traditional PEG is a linear polymer with chemically active hydroxyl groups at both ends [29]. PEG is biocompatible and is characterized by high water solubility [30]. It is readily cleared from the body, and it is widely used for drug conjugation.

PEGylation is a term used to describe a popular strategy that involves the conjugation of PEG with a therapeutic agent [31]. PEGylation is known to enhance the aqueous solubility of hydrophobic drugs, prolong circulation time, minimize nonspecific uptake, and achieve specific tumor targetability through the enhanced permeability and retention effect [29][32][33]. PEGs form a hydrated PEG layer, which resists the adsorption of serum proteins and phagocytic uptake. This effect has been called a stealth effect. The stealth effect of PEGylation improves the blood circulation half-lives of biopharmaceuticals as well as nanoparticles [34].

Furthermore, PEG shows a high solubility in organic solvents and, therefore, end-group modifications are relatively easy. When attached to drugs or carriers, it provides drugs with greater physical and thermal stability as well as prevents or reduces aggregation of the drugs in vivo and during storage, as a result of the steric hindrance and/or masking of charges [35].

PEG has limited conjugation capacity since it has only one terminal functional group at the end of the polymer chain (two in the case of modified PEG). This limitation is proposed to be overcome by coupling amino acids like aspartic acids and bicarboxylic amino acids to the PEG [32]. Another limitation of PEG is that it is non-biodegradable, resulting in possible accumulation in the body if the size of the nanoparticles is greater than the renal threshold. From a theoretical point of view, a biodegradable polymer would be more beneficial in applications, since difficulties in achieving complete excretion would be avoided, although other issues, such as the toxicity of degradation products and the limited shelf life, would need to be considered [35].

PEG has been known as a safe, inert, and non-immunogenic synthetic polymer. However, PEG-related immunological issues have received considerable attention [36][37][38][39][40][41]. Anti-PEG antibodies have been found in patients who were treated with PEGylated nonhuman enzymes. Furthermore, circulating anti-PEG antibodies have been found in healthy subjects and are thought to be induced by PEG-containing cosmetics and foods [34].

2.3. Poly-L-glutamic Acid (PGA)

PGA is made of naturally occurring l-glutamic acid connected through amide bonds as opposed to a nondegradable C-C backbone like other synthetic polymers that have been tested in clinical studies. Each repeating unit of l-glutamic acid contains a pendent-free γ-carboxyl group that is negatively charged at a neutral pH, making the polymer water soluble. The carboxyl groups also function as a means of attaching drugs. PGA is nontoxic and biodegradable which makes it a promising candidate for use as a carrier for the targeted administration of chemotherapy [42].

Electrostatic repulsion interactions between the negatively charged polymer and the relatively negatively charged surface of cells can limit its uptake by cells [43]. Nevertheless, the EPR effect, as well as the accumulation and retention of PGA–drug conjugates in solid tumors have been reported [42][44][45].

With increasing pH, PGA exhibits a conformational transition from a rod-like form in the α-helix state to a more random coil structure at the midway of pH 5.5, as revealed by a magnetic resonance study [46]. Therefore, PGA is expected to exist as a random coil at a neutral pH. The value of pH has a significant impact on the rate of PG's enzymatic breakdown [47][48]. Moreover, it was discovered that both the composition and the sequential distribution of co-monomers in the copolymer chains influenced the rate of degradation [49]. Additionally, the overall biodegradation of the PG polymer can be impacted by the conjugation of therapeutic molecules to PG. Degradation of the polymer backbone may or may not result in the release of free drug, depending on the type of bonds utilized to attach the drug molecules to PGA [50].

The degradability of PGA and its derivatives has been examined in several investigations using isolated tissue lysosomal enzymes [42][51][52]. In comparison to poly(l-aspartic acid) and poly(d-glutamic acid), PGA was found to be more vulnerable to lysosomal breakdown, and the breakdown of PG results in the formation of monomeric l-glutamic acid [42].

2.4. Poly(Lactic-co-glycolic Acid) (PLGA)

PLGA is a biocompatible polyester that is produced by a catalyzed ring-opening copolymerization of lactic acid (LA) and glycolic acid (GA) [53]. PLGA is a semicrystalline material with hydrophobic properties, and it degrades readily under physiological conditions. While PGA is a crystalline hydrophilic polymer with low water solubility and a fast degradation rate under physiological conditions, PLA is a stiff and hydrophobic polymer with low mechanical strength. As a copolymer of both, PLGA combines the intrinsic properties of its constitutional monomers where the LA/GA ratio strongly affects its degradation rate. For example, with an increase in the LA/GA ratio, the overall PLGA hydrophobicity increases, which leads to lower degradation and thus a slower drug release rate [54]. PLGA decomposes into non-toxic products (H_2O and CO_2) that are eliminated from the body [31]. In vivo, it degrades through hydrolysis of the ester bonds to its monomeric anions (LA and GA). While L-LA is converted into CO_2, which is excreted through the lungs, and it is converted to pyruvate, that then enters the Krebs cycle, D-LA is not further metabolized before excretion. GA, on the other hand, is either directly excreted through the renal system or can be oxidized to glyoxylate, which is afterward further converted into glycine, serine, and pyruvate. The latter can again enter the Krebs cycle and is metabolized into CO_2 and H_2O [55][56][57].

2.5. Poly(α-l-lysine) (PLL)

PLL is a water-soluble cationic biopolymer, built from monomeric unit α-l-lysine. Traditionally, three polymerization approaches are employed for PLL synthesis: solid-phase peptide synthesis (SPPS) [58][59], ring-opening polymerization (ROP) [60][61], and chemo-enzymatic synthesis [58].

PLLs use was proposed widely in various biomedical domains and the pharmaceutical field due to their inherent properties such as non-antigenicity, biocompatibility, and biodegradability. The building monomer, α-l-lysine, is one of the 20 naturally occurring amino acids. It is believed to be essential for eukaryotes and prokaryotes and plays critical roles in biological processes, including injury recovery and protein functions [58].

Under physiological conditions, PLL is positively charged due to the protonation of primary amino groups. PLL was developed as a functional biomedical material where the activity originates predominantly from this cationic property. Based on the electrostatic interactions between the positively charged PLL and the negatively charged components, PLLs have been investigated for application in nanocarrier synthesis, coating materials, and bacterial biofilm dispersal/membrane disruption [58].

On the other hand, hemolysis and cytotoxicity resulting from interactions between cationic PLL and the anionic membranes of red blood cells and vascular endothelial cells are the main concern in PLL biomedical applications. The cytotoxicity of PLL depends strongly on its molecular weight. PLLs with high molecular weight are more

deleterious to both mitochondrial oxidative phosphorylation and glycolytic activity, leading to significant intracellular ATP depletion and initiating necrotic-type cell death [58][62].

PLLs are classified as hydrophilic biopolymers because of their good water solubility. The presence of alkyl groups in their side chains results in the amphiphilicity of PLLs, which is neglected. Notably, PLLs can fold into a variety of secondary structures such as α-helical, β-sheet, and random coil based on hydrogen bonding and electrostatic interactions among their backbones and side chains. This secondary structure is frequently influenced by environmental stimuli, including pH, temperature, solvent variations, and surfactants [63][64][65], and results in different hydrophobicity [58].

References

1. Deng, S.; Gigliobianco, M.R.; Censi, R.; Di Martino, P. Polymeric Nanocapsules as Nanotechnological Alternative for Drug Delivery System: Current Status, Challenges and Opportunities. Nanomaterials 2020, 10, 847.
2. Blanco, E.; Shen, H.; Ferrari, M. Principles of Nanoparticle Design for Overcoming Biological Barriers to Drug Delivery. Nat. Biotechnol. 2015, 33, 941–951.
3. Gaumet, M.; Vargas, A.; Gurny, R.; Delie, F. Nanoparticles for Drug Delivery: The Need for Precision in Reporting Particle Size Parameters. Eur. J. Pharm. Biopharm. 2008, 69, 1–9.
4. Wang, Z.; Sun, C.; Wang, R. Macrocycle-Surfaced Polymer Nanocapsules: An Emerging Paradigm for Biomedical Applications. Bioconj. Chem. 2022, 33, 2254–2261.
5. Karabasz, A.; Bzowska, M.; Szczepanowicz, K. Biomedical Applications of Multifunctional Polymeric Nanocarriers: A Review of Current Literature. IJN 2020, 15, 8673–8696.
6. Trindade, I.C.; Pound-Lana, G.; Pereira, D.G.S.; de Oliveira, L.A.M.; Andrade, M.S.; Vilela, J.M.C.; Postacchini, B.B.; Mosqueira, V.C.F. Mechanisms of Interaction of Biodegradable Polyester Nanocapsules with Non-Phagocytic Cells. Eur. J. Pharm. Sci. 2018, 124, 89–104.
7. Teixeira, M.; Alonso, M.J.; Pinto, M.M.M.; Barbosa, C.M. Development and Characterization of PLGA Nanospheres and Nanocapsules Containing Xanthone and 3-Methoxyxanthone. Eur. J. Pharm. Biopharm. 2005, 59, 491–500.
8. Guterres, S.S.; Alves, M.P.; Pohlmann, A.R. Polymeric Nanoparticles, Nanospheres and Nanocapsules, for Cutaneous Applications. Drug Target. Insights 2007, 2, 117739280700200002.
9. McMahon, M.T.; Bulte, J.W.M. Two Decades of Dendrimers as Versatile MRI Agents: A Tale with and without Metals. WIREs Nanomed. Nanobiotechnol. 2018, 10, e1496.
10. Khemtong, C.; Kessinger, C.W.; Gao, J. Polymeric Nanomedicine for Cancer MR Imaging and Drug Delivery. Chem. Commun. 2009, 24, 3497–3510.

11. Sutton, D.; Nasongkla, N.; Blanco, E.; Gao, J. Functionalized Micellar Systems for Cancer Targeted Drug Delivery. Pharm. Res. 2007, 24, 1029–1046.
12. Torchilin, V.P. Multifunctional Nanocarriers. Adv. Drug Deliv. Rev. 2006, 58, 1532–1555.
13. Discher, D.E.; Ahmed, F. Polymersomes. Annu. Rev. Biomed. Eng. 2006, 8, 323–341.
14. Mora-Huertas, C.E.; Fessi, H.; Elaissari, A. Polymer-Based Nanocapsules for Drug Delivery. Int. J. Pharm. 2010, 385, 113–142.
15. Shastri, V.P. Non-Degradable Biocompatible Polymers in Medicine: Past, Present and Future. Curr. Pharm. Biotechnol. 2003, 4, 331–337.
16. Almeida, J.P.M.; Chen, A.L.; Foster, A.; Drezek, R. In Vivo Biodistribution of Nanoparticles. Nanomedicine 2011, 6, 815–835.
17. Ali, I.; Alsehli, M.; Scotti, L.; Tullius Scotti, M.; Tsai, S.-T.; Yu, R.-S.; Hsieh, M.F.; Chen, J.-C. Progress in Polymeric Nano-Medicines for Theranostic Cancer Treatment. Polymers 2020, 12, 598.
18. Dash, T.K.; Konkimalla, V.B. Poly-ϵ-Caprolactone Based Formulations for Drug Delivery and Tissue Engineering: A Review. J. Control. Release 2012, 158, 15–33.
19. Mondal, D.; Griffith, M.; Venkatraman, S.S. Polycaprolactone-Based Biomaterials for Tissue Engineering and Drug Delivery: Current Scenario and Challenges. Int. J. Polym. Mater. Polym. Biomater. 2016, 65, 255–265.
20. Palaniappan, R.; Korang-Yeboah, M.; Gorantla, Y.; Paulos, S.; Sharma, P.; Chaudhary, J. Polycaprolactone/Maltodextrin Nanocarrier for Intracellular Drug Delivery: Formulation, Uptake Mechanism, Internalization Kinetics, and Subcellular Localization. Int. J. Nanomed. 2015, 10, 4763–4781.
21. Woodruff, M.A.; Hutmacher, D.W. The Return of a Forgotten Polymer—Polycaprolactone in the 21st Century. Prog. Polym. Sci. 2010, 35, 1217–1256.
22. Wei, X.; Gong, C.; Gou, M.; Fu, S.; Guo, Q.; Shi, S.; Luo, F.; Guo, G.; Qiu, L.; Qian, Z. Biodegradable Poly(ε-Caprolactone)–Poly(Ethylene Glycol) Copolymers as Drug Delivery System. Int. J. Pharm. 2009, 381, 1–18.
23. Pitt, C.G.; Chasalow, F.I.; Hibionada, Y.M.; Klimas, D.M.; Schindler, A. Aliphatic Polyesters. I. The Degradation of Poly(ϵ-Caprolactone) in Vivo. J. Appl. Polym. Sci. 1981, 26, 3779–3787.
24. Woodward, S.C.; Brewer, P.S.; Moatamed, F.; Schindler, A.; Pitt, C.G. The Intracellular Degradation of Poly(ε-Caprolactone). J. Biomed. Mater. Res. 1985, 19, 437–444.
25. Sun, H.; Mei, L.; Song, C.; Cui, X.; Wang, P. The in Vivo Degradation, Absorption and Excretion of PCL-Based Implant. Biomaterials 2006, 27, 1735–1740.

26. Vert, M. Degradable and Bioresorbable Polymers in Surgery and in Pharmacology: Beliefs and Facts. J. Mater. Sci. Mater. Med. 2009, 20, 437–446.
27. Dwivedi, R.; Kumar, S.; Pandey, R.; Mahajan, A.; Nandana, D.; Katti, D.S.; Mehrotra, D. Polycaprolactone as Biomaterial for Bone Scaffolds: Review of Literature. J. Oral Biol. Craniofac. Res. 2020, 10, 381–388.
28. Espinoza, S.M.; Patil, H.I.; San Martin Martinez, E.; Casañas Pimentel, R.; Ige, P.P. Poly-ε-Caprolactone (PCL), a Promising Polymer for Pharmaceutical and Biomedical Applications: Focus on Nanomedicine in Cancer. Int. J. Polym. Mater. Polym. Biomater. 2020, 69, 85–126.
29. Shi, L.; Zhang, J.; Zhao, M.; Tang, S.; Cheng, X.; Zhang, W.; Li, W.; Liu, X.; Peng, H.; Wang, Q. Effects of Polyethylene Glycol on the Surface of Nanoparticles for Targeted Drug Delivery. Nanoscale 2021, 13, 10748–10764.
30. Tohamy, H.-A.S.; El-Sakhawy, M.; Kamel, S. Carboxymethyl Cellulose-Grafted Graphene Oxide/Polyethylene Glycol for Efficient Ni(II) Adsorption. J. Polym. Environ. 2021, 29, 859–870.
31. Manandhar, S.; Sjöholm, E.; Bobacka, J.; Rosenholm, J.M.; Bansal, K.K. Polymer-Drug Conjugates as Nanotheranostic Agents. JNT 2021, 2, 63–81.
32. Banerjee, S.S.; Aher, N.; Patil, R.; Khandare, J. Poly(Ethylene Glycol)-Prodrug Conjugates: Concept, Design, and Applications. J. Drug Deliv. 2012, 2012, 103973.
33. Suk, J.S.; Xu, Q.; Kim, N.; Hanes, J.; Ensign, L.M. PEGylation as a Strategy for Improving Nanoparticle-Based Drug and Gene Delivery. Adv. Drug Deliv. Rev. 2016, 99, 28–51.
34. Shiraishi, K.; Yokoyama, M. Toxicity and Immunogenicity Concerns Related to PEGylated-Micelle Carrier Systems: A Review. Sci. Technol. Adv. Mater. 2019, 20, 324–336.
35. Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U.S. Poly(Ethylene Glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. Angew. Chem. Int. Ed. 2010, 49, 6288–6308.
36. Hershfield, M.S.; Ganson, N.J.; Kelly, S.J.; Scarlett, E.L.; Jaggers, D.A.; Sundy, J.S. Induced and Pre-Existing Anti-Polyethylene Glycol Antibody in a Trial of Every 3-Week Dosing of Pegloticase for Refractory Gout, Including in Organ Transplant Recipients. Arthritis Res. Ther. 2014, 16, R63.
37. Hoang Thi, T.T.; Pilkington, E.H.; Nguyen, D.H.; Lee, J.S.; Park, K.D.; Truong, N.P. The Importance of Poly(Ethylene Glycol) Alternatives for Overcoming PEG Immunogenicity in Drug Delivery and Bioconjugation. Polymers 2020, 12, 298.
38. Longo, N.; Harding, C.O.; Burton, B.K.; Grange, D.K.; Vockley, J.; Wasserstein, M.; Rice, G.M.; Dorenbaum, A.; Neuenburg, J.K.; Musson, D.G.; et al. Single-Dose, Subcutaneous Recombinant Phenylalanine Ammonia Lyase Conjugated with Polyethylene Glycol in Adult Patients with Phenylketonuria: An Open-Label, Multicentre, Phase 1 Dose-Escalation Trial. Lancet 2014, 384, 37–44.

39. Garay, R.P.; El-Gewely, R.; Armstrong, J.K.; Garratty, G.; Richette, P. Antibodies against Polyethylene Glycol in Healthy Subjects and in Patients Treated with PEG-Conjugated Agents. Expert Opin. Drug Deliv. 2012, 9, 1319–1323.
40. Ganson, N.J.; Povsic, T.J.; Sullenger, B.A.; Alexander, J.H.; Zelenkofske, S.L.; Sailstad, J.M.; Rusconi, C.P.; Hershfield, M.S. Pre-Existing Anti–Polyethylene Glycol Antibody Linked to First-Exposure Allergic Reactions to Pegnivacogin, a PEGylated RNA Aptamer. J. Allergy Clin. Immunol. 2016, 137, 1610–1613.e7.
41. Lipsky, P.E.; Calabrese, L.H.; Kavanaugh, A.; Sundy, J.S.; Wright, D.; Wolfson, M.; Becker, M.A. Pegloticase Immunogenicity: The Relationship between Efficacy and Antibody Development in Patients Treated for Refractory Chronic Gout. Arthritis Res. Ther. 2014, 16, R60.
42. Li, C. Poly(l-Glutamic Acid)–Anticancer Drug Conjugates. Adv. Drug Deliv. Rev. 2002, 54, 695–713.
43. Drobník, J.; Rypáček, F. Soluble Synthetic Polymers in Biological Systems. In Polymers in Medicine; Advances in Polymer Science; Springer: Berlin/Heidelberg, Germany, 1984; Volume 57, pp. 1–50. ISBN 978-3-540-12796-3.
44. Li, C.; Ke, S.; Wu, Q.-P.; Tansey, W.; Hunter, N.; Buchmiller, L.M.; Milas, L.; Charnsangavej, C.; Wallace, S. Potentiation of Ovarian OCa-1 Tumor Radioresponse by Poly (L-Glutamic Acid)-Paclitaxel Conjugate. Int. J. Radiat. Oncol. Biol. Phys. 2000, 48, 1119–1126.
45. Milas, L.; Mason, K.A.; Hunter, N.; Li, C.; Wallace, S. Poly(L-Glutamic Acid)-Paclitaxel Conjugate Is a Potent Enhancer of Tumor Radiocurability. Int. J. Radiat. Oncol. Biol. Phys. 2003, 55, 707–712.
46. Tsutsumi, A.; Perly, B.; Forchioni, A.; Chachaty, C. A Magnetic Resonance Study of the Segmental Motion and Local Conformations of Poly(L-Glutamic Acid) in Aqueous Solutions. Macromolecules 1978, 11, 977–986.
47. Miller, W.G. Degradation of Poly-α,L-Glutamic Acid. I. Degradation of High Molecular Weight PGA by Papain. J. Am. Chem. Soc. 1961, 83, 259–265.
48. Miller, W.G. Degradation of Synthetic Polypeptides. II. Degradation of Poly-α,L-Glutamic Acid by Proteolytic Enzymes in 0.20 M Sodium Chloride. J. Am. Chem. Soc. 1964, 86, 3913–3918.
49. Hayashi, T.; Iwatsuki, M. Biodegradation of Copoly(L-Aspartic Acid/L-Glutamic Acid) in Vitro. Biopolymers 1990, 29, 549–557.
50. Hoes, C.J.T.; Potman, W.; Van Heeswijk, W.A.R.; Mud, J.; De Grooth, B.G.; Greve, J.; Feijen, J. Optimization of Macromolecular Prodrugs of the Antitumor Antibiotic Adriamycin. J. Control. Release 1985, 2, 205–213.

51. Chiu, H.-C.; Kopečková, P.; Deshmane, S.S.; Kope?ek, J. Lysosomal Degradability of Poly(α-Amino Acids). J. Biomed. Mater. Res. 1997, 34, 381–392.
52. Mccormick-Thomson, L.A.; Duncan, R. Poly(Amino Acid) Copolymers as a Potential Soluble Drug Delivery System. 1: Pinocytic Uptake and Lysosomal Degradation Measured In Vitro. J. Bioact. Compat. Polym. 1989, 4, 242–251.
53. Dechy-Cabaret, O.; Martin-Vaca, B.; Bourissou, D. Controlled Ring-Opening Polymerization of Lactide and Glycolide. Chem. Rev. 2004, 104, 6147–6176.
54. Xu, Y.; Kim, C.-S.; Saylor, D.M.; Koo, D. Polymer Degradation and Drug Delivery in PLGA-Based Drug-Polymer Applications: A Review of Experiments and Theories: Review on Biodegradation and Drug Delivery from PLGA Polymers. J. Biomed. Mater. Res. 2017, 105, 1692–1716.
55. Danhier, F.; Ansorena, E.; Silva, J.M.; Coco, R.; Le Breton, A.; Préat, V. PLGA-Based Nanoparticles: An Overview of Biomedical Applications. J. Control. Release 2012, 161, 505–522.
56. Silva, A.T.C.R.; Cardoso, B.C.O.; Silva, M.E.S.R.E.; Freitas, R.F.S.; Sousa, R.G. Synthesis, Characterization, and Study of PLGA Copolymer in Vitro Degradation. JBNB 2015, 6, 8–19.
57. Rezvantalab, S.; Drude, N.I.; Moraveji, M.K.; Güvener, N.; Koons, E.K.; Shi, Y.; Lammers, T.; Kiessling, F. PLGA-Based Nanoparticles in Cancer Treatment. Front. Pharmacol. 2018, 9, 1260.
58. Zheng, M.; Pan, M.; Zhang, W.; Lin, H.; Wu, S.; Lu, C.; Tang, S.; Liu, D.; Cai, J. Poly(α-l-Lysine)-Based Nanomaterials for Versatile Biomedical Applications: Current Advances and Perspectives. Bioact. Mater. 2021, 6, 1878–1909.
59. Jaradat, D.M.M. Thirteen Decades of Peptide Synthesis: Key Developments in Solid Phase Peptide Synthesis and Amide Bond Formation Utilized in Peptide Ligation. Amino Acids 2018, 50, 39–68.
60. Deming, T.J. Synthesis of Side-Chain Modified Polypeptides. Chem. Rev. 2016, 116, 786–808.
61. Shen, Y.; Fu, X.; Fu, W.; Li, Z. Biodegradable Stimuli-Responsive Polypeptide Materials Prepared by Ring Opening Polymerization. Chem. Soc. Rev. 2015, 44, 612–622.
62. Hall, A.; Wu, L.-P.; Parhamifar, L.; Moghimi, S.M. Differential Modulation of Cellular Bioenergetics by Poly(l-Lysine)s of Different Molecular Weights. Biomacromolecules 2015, 16, 2119–2126.
63. Semenyshyn, R.; Hentschel, M.; Stanglmair, C.; Teutsch, T.; Tarin, C.; Pacholski, C.; Giessen, H.; Neubrech, F. In Vitro Monitoring Conformational Changes of Polypeptide Monolayers Using Infrared Plasmonic Nanoantennas. Nano Lett. 2019, 19, 1–7.
64. Rahikkala, A.; Junnila, S.; Vartiainen, V.; Ruokolainen, J.; Ikkala, O.; Kauppinen, E.; Raula, J. Polypeptide-Based Aerosol Nanoparticles: Self-Assembly and Control of Conformation by Solvent and Thermal Annealing. Biomacromolecules 2014, 15, 2607–2615.

65. Chen, J.; Zou, X. Self-Assemble Peptide Biomaterials and Their Biomedical Applications. Bioact. Mater. 2019, 4, 120–131.