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PLGA's Plight and the Role of Stealth Surface Modification Strategies in Its Use for Intravenous Particulate Drug Delivery

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Numerous human disorders can benefit from targeted, intravenous (IV) drug delivery. Polymeric nanoparticles have been designed to undergo systemic circulation and deliver their therapeutic cargo to target sites in a controlled manner. Poly(lactic-*co*-glycolic) acid (PLGA) is a particularly promising biomaterial for designing intravenous drug carriers due to its biocompatibility, biodegradability, and history of clinical success across other routes of administration. Despite these merits, PLGA remains markedly absent in clinically approved IV drug delivery formulations. A prominent factor in PLGA particles' inability to succeed intravenously may lie in the hydrophobic character of the polyester, leading to the adsorption of serum proteins (i.e., opsonization) and a cascade of events that end in their premature clearance from the bloodstream. PEGylation, or surface-attached polyethylene glycol chains, is a common strategy for shielding particles from opsonization. Polyethylene glycol (PEG) continues to be regarded as the ultimate "stealth" solution despite the lack of clinical progress of PEGylated PLGA carriers. This review reflects on some of the reasons for the clinical failure of PLGA, particularly the drawbacks of PEGylation, and highlights alternative surface coatings on PLGA particles. Ultimately, a new approach will be needed to harness the potential of PLGA nanoparticles and allow their widespread clinical adoption.

1. Introduction

Intravenous administration of drug carriers remains a relevant platform for treating various ailments from cardiovascular disease to cancer to blood disorders. The ability to inject a therapeutic directly into the bloodstream to actively (i.e., via cell-specific ligands) or passively (i.e., via endothelial permeability) target a diseased site not only enhances the pharmaceutical bioavailability but also reduces potential side effects.[1,2] By providing direct access to the circulatory system, IV administration allows the fastest pathway for drug delivery vehicles to move throughout the body.[3,4]

Of the possible biomaterial candidates for designing an intravenous drug delivery carrier, biodegradable synthetic polymers such as PLGA are ideal candidates. Compared to its drug delivery contemporaries (i.e., dendrimers and liposomes), PLGA exhibits lower toxicity, higher customizability, greater stability, and the unique ability of sustained drug release.[5] Presently, PLGA is the most widely investigated synthetic biodegradable polymer for particulate drug delivery systems.[6,7] With a well-characterized degradation pattern and high biocompatibility, PLGA has also been used in a plethora of clinically approved medical devices, such as grafts, sutures, and scaffolds. Nonetheless, a thorough investigation of its commercial history and clinical application reveals that the biopolymer has not yet found a significant presence in *intravenous* drug delivery systems.[8]

The design of successful IV drug delivery vehicles comes with a unique set of challenges, and certain physiochemical properties of PLGA have put it at odds with intravenous transport. Fundamentally, the efficacy of any drug delivery vehicle administered intravenously depends upon its ability to circulate for extended periods without severe alteration of its biological identity.[9] However, PLGA, by virtue of its hydrophobic character and negative surface charge, experiences opsonization immediately following its injection into the bloodstream. Blood is a complex fluid with an innate defense mechanism that seeks to protect the host from pathogens. Opsonins are serum proteins whose primary role is to mark the particles as "foreign" intruders by adsorbing onto their surface. Opsonization involves a variety of serum proteins, e.g.,

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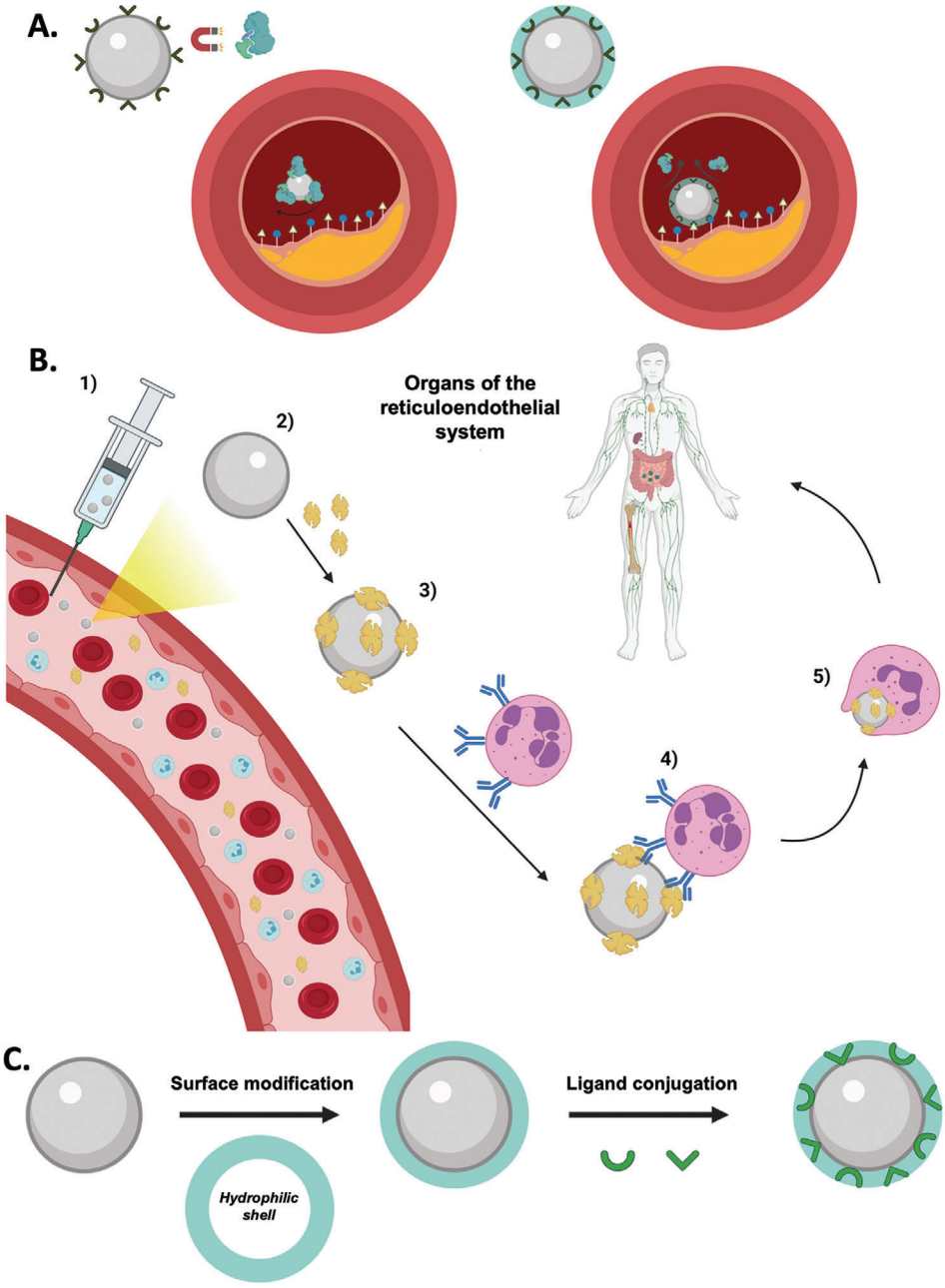

Figure 1. A) (Atherosclerotic model) The targeting efficacy of bare particles to the diseased endothelium is obstructed by opsonins while that of hydrated carriers is preserved in the presence of opsonins. B) The opsonization and phagocytosis cascade of model drug carriers immediately following intravenous injection. C) Bare particle surface modified with hydrophilic coating and subsequently conjugated with targeting ligands.

apolipoproteins, fibrinogen, and complement immunoglobulins. As soon as the nanoparticles enter the bloodstream, the proteins with the highest mobility approach the injected particles.[10] The initial protein corona that forms is known as the soft corona. It consists of the less abundant and lower affinity proteins. With time, some of these proteins are replaced by the relatively high-affinity proteins leading to a new nanoparticle–protein complex known as the hard corona. This mechanism, known as opsonization, will be set off within minutes after a PLGA particle or other unmodified hydrophobic surface enters the whole blood environment. Overall, opsonization can serve to impede particulate drug delivery by evoking two major effects: 1) Obstructed margination (**Figure 1**A, part one), wherein opsonins mask targeting ligands or other active markers on the particle exterior, which interferes with particles' ability to localize and adhere, or marginate, to the target site,[11] and 2) Premature immune clearance, which ultimately leads to therapeutic agents being released at off-target sites or no release at all (Figure 1B). This latter outcome occurs

because the presence of opsonins on a particle surface signals circulating neutrophils and monocytes to approach and initiate a cascade of events, leading to phagocytosis (cellular ingestion).

Whether its margination (i.e., localization and adhesion to the vascular wall) or immunogenicity being assessed, the particle *surface* provides the primary interface to govern its interactions with the whole blood environment and, in turn, regulate its fate in vivo.[12] Therefore, much of drug carrier design has focused on strategies to mitigate opsonization, with non-fouling approaches dominating for the last three to four decades. Similar to anti-microbial and marine applications, intravenous drug delivery necessitates the design of surfaces that are resistant to opsonins to alleviate the issues of ligand masking, immune clearance, and to ensure margination to the target site. Given the proper surface coating, particles can maintain a level of invisibility to the host's physiological clearance mechanisms and remain inert to the various blood components. The traditional strategy for achieving this "stealth" effect on polymeric particles is "PEGylation," or attaching polyethylene glycol (PEG) chains on the surface of the particle.[13,14] PEG serves as a neutrally charged, hydrophilic layer with the ability to undergo several polymer conformations to ward off neighboring protein molecules. Although PEG has shown considerable promise in shielding carriers from specific classes of phagocytes and lengthening residence time in vivo, an increasing number of reports continue to expose its limitations. One current example is the low molecular weight PEG excipients that were incorporated to improve the steric stabilization of the lipid nanoparticle mRNA formulation for both the Pfizer/BioNTech and Moderna COVID-19 vaccines linked to the severe anaphylactic episodes that two patients experienced a few days into the UK Pfizer vaccination campaign.[15] Although these formulations were intramuscular, the same intolerances occur when PEG-based therapeutics are administered in the vascular space.[16] Besides allergy-related hypersensitivity reactions, many reports have implicated PEG in organ dysfunction, accelerated blood clearance, and complement activation.[17,18] Thus, researchers must continue to explore plausible PEG alternatives for nanoparticle formulation for intravenous drug delivery.

This review seeks to provide a comprehensive overview of stealth coatings that have been tried and assessed solely in relation to *PLGA*-based particulate drug carriers. We particularly focus on intravenous (IV) PLGA particle formulations for drug delivery, given that PLGA still lacks commercial presence in this space despite the plethora of publications reporting positive effects in animal models. Several other reputable works have summarized the advantages and disadvantages of PEG coatings across various particle systems, including polystyrene, gold, iron, mesoporous silica, peptides, proteins, micelles, hydrogels, and liposomal nanoparticles, and subsequently presented prospective alternative coatings.[19–23] We offer a similar summary for PLGA here and strive to emphasize often overlooked information, providing a comprehensive overview of stealth coatings that have been explored solely in relation to PLGA nano-drug delivery systems. See **Figure 2** for a summary of the various compounds discussed. Overall, we seek to reflect on the potential of PLGA particles as controlled release vehicles and invite our colleagues to look beyond PEG to develop novel viable alternatives to enable success in IV-based clinical translation.

2. The Clinical Backdrop of Intravenous PLGA Drug Delivery Formulations

Over three decades of research have gone into developing PLGA-based drug delivery formulations (see **Figure 3** for an exact timeline). Since the execution of the first human clinical trial in 1981, at least 12 injectable PLGA microparticle (MP) formulations have gained approval by both the United States Food & Drug Administration (US FDA) and the European Medical Agency (EMA).[8] However, the routes of administration for these formulations have been limited to intramuscular, subcutaneous, periodontal, and intraarticular. To date, there are no known IV-based PLGA NP or MP formulations approved for clinical use. This scarcity is unique to PLGA as its drug delivery contemporary, liposomes, are more abundant in clinically approved IV formulations and other administration routes.[24]

Some may point to Genexol®-PM to highlight the clinical success of an IV-delivered polymer drug carrier. However, it is worth highlighting that this cancer therapeutic, approved in South Korea for clinical use, is a block copolymer micellar formulation, solubilizing anticancer drug paclitaxel.[25] Within the Genexol PM® micelle, the hydrophobic portion of the block copolymer is composed of PLA, while the hydrophilic block is PEG. PLA is the sister polymer of PLGA, and although not equivalent, it shares PLGA's attributes of biodegradability, a hydrophobic polyester backbone, and widespread use in biomedical applications. PLA also contributes to the majority of PLGA's hydrophobic character. However, given polymeric micelles are supramolecular core/shell nanostructures made from the self-assembly of amphiphilic block copolymers,[26] the entirety of the hydrophilic block is presented at the particle surface, likely diminishing PLA's impact on Genexol®-PM circulation and clearance.

One can argue that a possible explanation for this discrepancy between PLGA's and liposomes' clinical utility is merely chronology, i.e., liposomes have been under study for much longer and thus have a head start in undergoing the regulatory process (see Figure 3, liposomal timeline). Indeed, liposomes were the first drug delivery systems successfully translated into clinical use. However, while it is true that liposomes were described in the literature as early as 1964 by British hematologist Alec D Bangham,[27] the homopolymers of PLGA, PLA, and poly(glycolic acid) had already begun development in the late 1950s. The first successful milestone in liposome-based products took place in 1995 with the introduction of Doxil® to the U.S. market to treat patients with ovarian cancer and AIDS-related Kaposi's sarcoma. However, Decapeptyl® SR, the first product based on PLGA microspheres, was granted FDA approval in 1989 prior to Doxil's debut (see Figure 3).[28] The close timing of their developments, as well as the fact that 11 of the 14 clinically approved liposomal formulations are intravenous, indicates that PLGA's slow development in IV form is not merely an issue of chronology. One could then ask, why have liposomes gained widespread IV use and not PLGA, a seemingly more versatile polymer?

2.1. Elucidating Particle Characteristics Conducive to Intravenous Drug Delivery

Chronology aside, it is important to acknowledge that although liposomes and PLGA drug delivery vehicles are functionally the

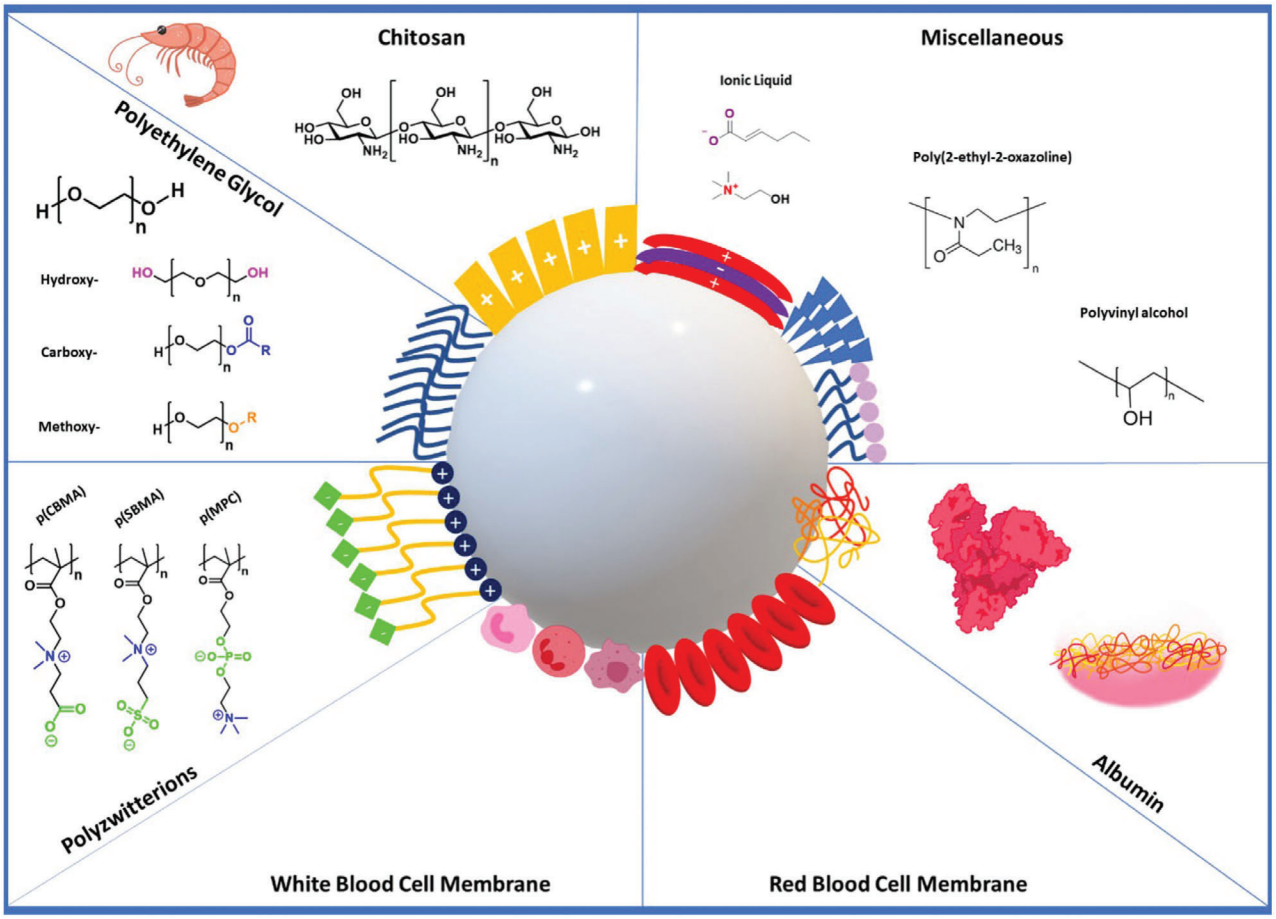

Figure 2. Contemporary Stealth Surface Modifications for Improved PLGA Particulate Drug Delivery.

same—both serve to prolong and, at times, target their pharmaceutical cargo to a site of action—they are in most other aspects, and especially compositionally, different. See **Figure 4** for a more detailed presentation of their similarities and differences. Liposomes are globular-like vesicles consisting of at least one phospholipid bilayer membrane, a defining feature that closely mimics cellular membranes.[29,30] They can be fabricated using either natural or synthetic lipids or a combination of the two. PLGA, on the other hand, is always synthetic, a product of ring-opening polymerization of the cyclic dimers of lactic acid and glycolic acid. The closest PLGA comes to having a biomimetic nature is that upon hydrolytic degradation, its byproducts, lactic and glycolic acid, are readily processed as part of the body's natural metabolic pathways.

Thus, a more plausible explanation for the discrepancy in the number of approved IV formulations between the two drug delivery vehicles lies in the *hydrophobic* character intrinsic to the PLGA biopolymer. Several publications have suggested that surface hydrophobicity is a critical factor in the phagocytic uptake of particulate matter by the mononuclear phagocytic system (MPS).[31–34] This clearance process is directly linked to opsonization, where opsonins in plasma coat the surface of particulate matter, targeting it as a foreign entity primed for recognition via MPS. As a polyester with several methyl groups sticking out along the backbone, PLGA is involved in several hydrophobic interactions and repels water. According to the Whitesides' Rules, any protein-resistant or non-fouling surface should be characterized by the following two traits: 1) polar (hydrophilic) functional groups and hydrogen bond acceptor groups, and 2) no hydrogen bond donor groups or net charge.[35] The zeta potential of bare PLGA nano- and micro-particles hovers around −20 to −40 mV, and the ester and ketone groups do not qualify as hydrophilic groups.[36] Additionally, the carboxylic acid and hydroxyl end groups act as hydrogen bond donors.

By virtue of their self-assembly in aqueous media, liposomes and micelles have an abundance of hydrophilic end groups pointing outward to form a hydrated surface.[29,37] Common lipids used to form the membrane are naturally sourced and include egg or soybean phosphatidylcholine (PC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and cholesterol (Chol).[38] Both PC and DOPE are neutrally charged and act as hydrogen bond acceptors, thus satisfying two of Whitesides' rules. These characteristics combined with their biomimetic nature may help to explain why liposomes have had greater success in intravenous drug delivery than PLGA carriers.

Interestingly, few clinically approved intravenous liposomal formulations are PEGylated or even surface modified with other agents to prevent their uptake by the reticuloendothelial system

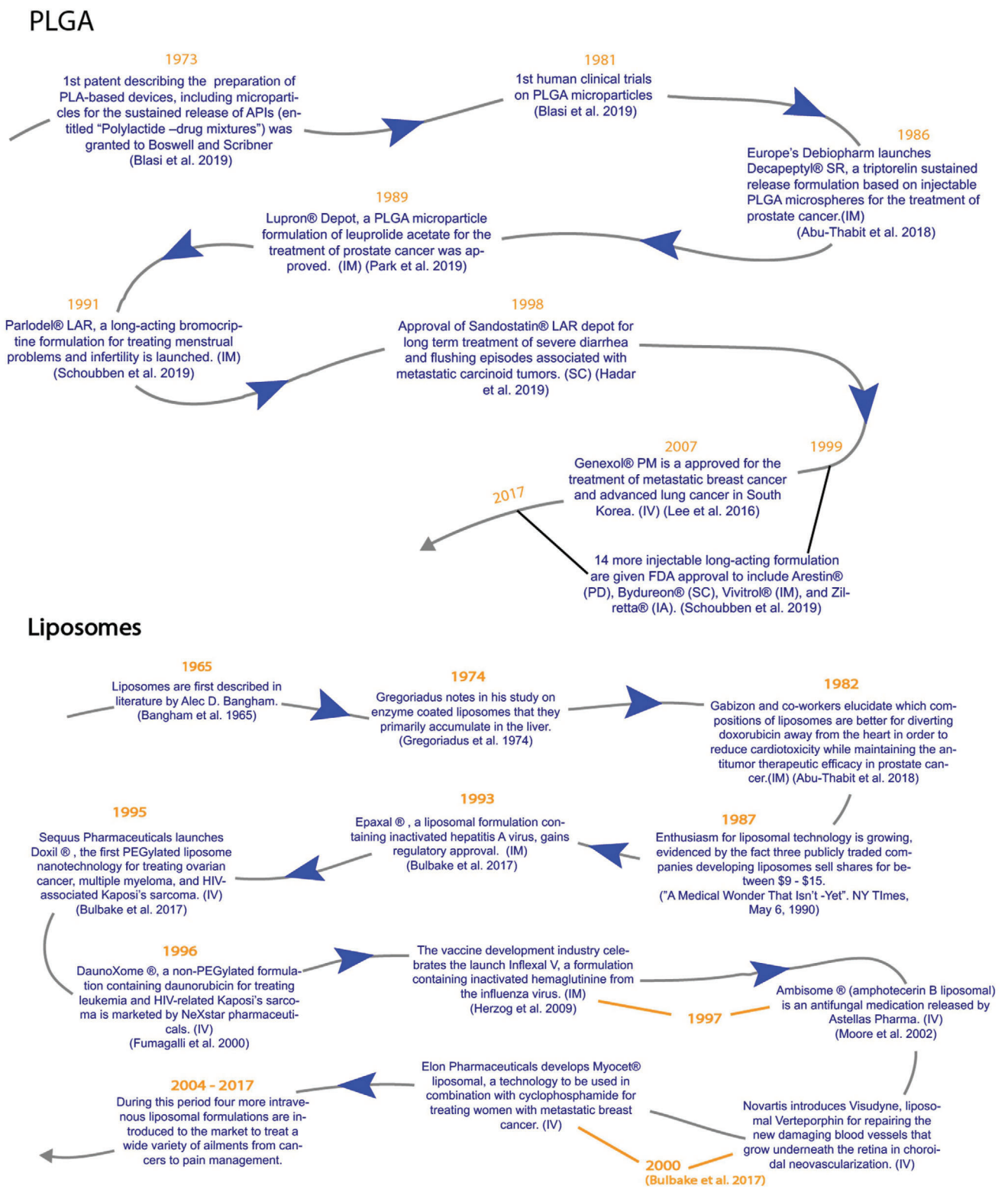

Figure 3. Timeline for clinical development of both PLGA/PLA Particle-Based therapeutics and liposomal formulations (not intended to be exhaustive). IM = intramuscular, SC = subcutaneous, PD = periodontal, IA = intraarticular, IV = intravenous.

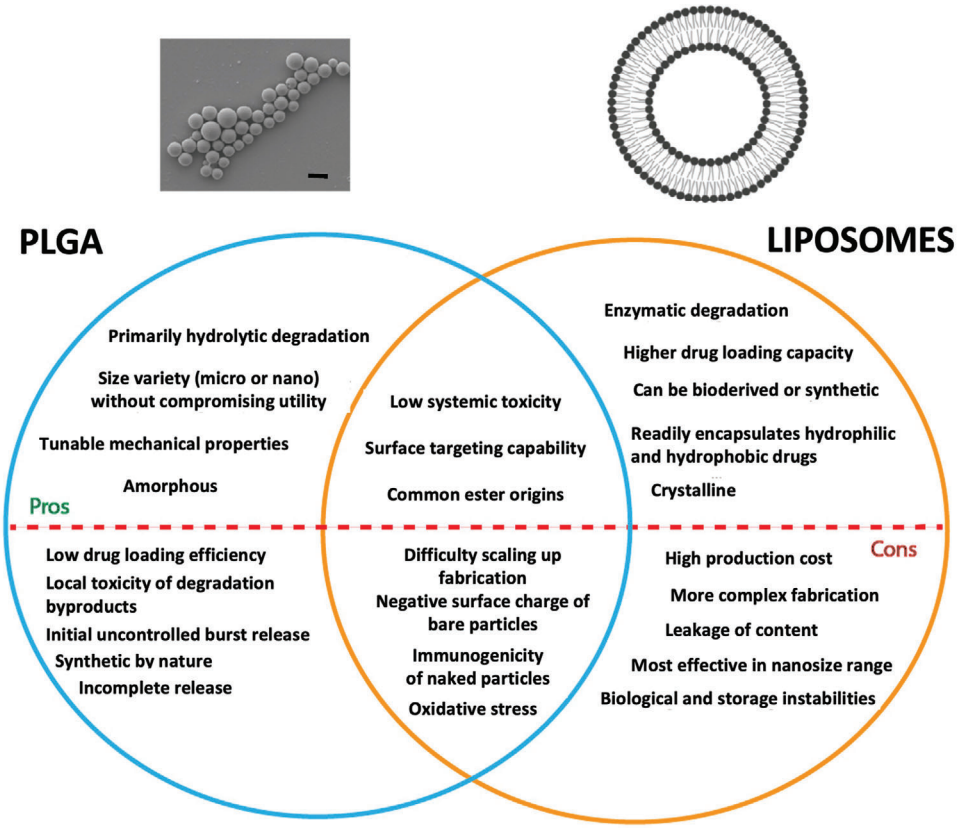

Figure 4. Venn diagram comparing the strengths and shortcomings impacting both PLGA and liposomal based drug delivery systems in intravenous applications. Left: PLGA microparticles (SEM micrograph from author's lab displaying general PLGA particle morphology—smooth, nonporous surface; scale bar = 1 micron). Right: liposomes having lipid bilayer membrane morphology. Pros of each particle type is written above the dotted red line, while cons are written below the dotted red line.

(RES). Of the intravenous liposome formulations in the clinic, only Doxil and Onivyde incorporate PEG in the formulation process, while the other eleven liposomes remain unmodified.[24] Although not liposomal, a lipid nanoparticle system, ONPATTRO®, also known as patisiran, incorporates PEG for IV delivery of silent interfering RNA (siRNA) for polyneuropathy treatment.[39] The COVID19 vaccines mentioned in Section 1 are lipid-based systems that also incorporate PEG; however, they exist in the tissue space being administered intramuscularly. The fact most *IV-administered* liposomes do not require any surface modification to have clinical success suggests caution against making sweeping generalizations about drug delivery systems because therapeutic performance is a material-specific phenomenon. Indeed, the lack of necessity for PEG coating on liposome-based drug carriers for IV delivery was noted as early as 1999 in a study by the National Taiwan University.[40] A direct comparison of PEGylated liposomal doxorubicin (Dox) and bare liposomal doxorubicin revealed that although the plasma AUC (Area Under the Curve) levels were twice as high for the former, the latter exhibited a doubly high tumor accumulation efficiency. Overall, the authors noted no difference in the therapeutic efficacy between both liposome groups; toxicity, tumor shrinkage, and survival were more or less similar. While PEG performed its intended function of extending the circulation time of Dox, the study implied that the steric barrier introduced by PEG hindered interactions with tumor cells.

Additional studies with liposomes have implied that selection of phospholipids with high transition temperatures (the temperature where the vesicle changes from the ordered gel phase to disordered liquid crystalline phase) is sufficient to prolong blood residence time without using PEG as an excipient.[41,42] These findings and the abundant amount of PEG-less, IV liposomal formulations suggest that PLGA may have a very different set of roadblocks towards IV administration, and comparisons to liposomes should not be a rigorous point of contention.

Similarly, while over a dozen clinical studies have demonstrated the previously mentioned micellar formulation, Genexol PM®, to exhibit a greater anticancer effect and lower toxicity than free Taxol, the precise benefit of having PEG in the formulation is not clear. Pre-clinical in vivo mice studies comparing Taxol with Genexol-PM® suggested that the latter demonstrated shorter plasma half-lives. Biodistribution of paclitaxel after administration of Genexol-PM® showed twofold to threefold higher levels in tissues including liver, spleen, kidneys, lungs, heart, and tumor compared to Taxol.[43] The fast clearance of the chemotherapeutic from the bloodstream was advantageous because less drug was available to cause the adverse side effect of myelosuppression. During Phase I trials of Genexol-PM®

conducted in South Korea, no acute hypersensitivity reactions were observed.[44] Overall, the therapeutic is producing the desired antitumor effect. However, the expected functionality of the PEG moieties to enhance circulation time and avoid premature clearance appears to be either lacking or ambiguous. Without a non-PEGylated micelle to compare against, it is unclear to what extent the PEG block brings longevity to the micelle beyond enhancing the solubility of the paclitaxel. Of note, Genexol-PM® represents a first of its class polymeric micelle formulation to gain clinical approval amidst any mode of administration,[45] and clinical trial for US FDA approval is ongoing.

2.2. Key Challenges to the Use of PLGA as a Drug Carrier

As stated previously, premature immune clearance and obstructed margination, i.e., vascular wall localization and adhesion, remain some of the greatest challenges to designing effective intravenous and vascular-targeted drug delivery vehicles. Both effects are consequences of opsonization. The probability of effective margination is greatly reduced when bare particles are introduced to the bloodstream because there exists no protection from circulating opsonins. Our published works have extensively shown the dire effects of a protein corona in diverting particles from the target site.[11,46,47] However, when particles are modified with hydrophilic, stealth moieties (Figure 1C), hydrophobic interactions with opsonins are minimized, leaving a greater surface area of targeting ligands exposed and available for their intended function (Figure 1A, part two). Figure 4 demonstrates a variety of factors besides hydrophobicity and opsonization that impede the progress of PLGA particles towards clinical IV administration. We will briefly review these two challenges in this section and offer the ways in which scholars are approaching these issues.

2.2.1. Hydrophilic Drug Entrapment

While hydrophobic drugs readily encapsulate into the PLGA core, their hydrophilic counterparts often require more complex modification techniques. During particle fabrication, small hydrophilic drugs tend to partition from the organic solvent phase to the surrounding aqueous media. This challenge may partially explain the absence of intravenous PLGA formulations in the clinic compared to liposomes, given that approximately 30–40% of drugs are hydrophilic[48] (e.g., doxorubicin hydrochloride) and the route of intravenous administration allows hydrophilic drugs to achieve 100% of bioavailability.[49] Liposomes, however, readily and simultaneously encapsulate opposite polarity drugs in a bilayer membrane.

A few strategies have been developed to address the entrapment disparity for PLGA. However, the level of precision in fabrication parameters required or the necessity of different additives does not yet match the ease of approach that comes with using liposomes. Espanol et al. utilized an ethyl acetate/methanol cosolvent system and systematically modified three parameters—drug/polymer ratio, surfactant concentration, and sonication time to achieve dual drug loading efficiencies ranging from 2.4% to 4.7%.[50] Narayanan et al. developed a multicompartmental nanoconstruct based on PLGA and milk protein casein to dually encapsulate paclitaxel in the core and epigallocatechin gallate (EGCG) in the shell.[51] By using a 1:10 ratio of EGCG:casein, hydrophilic drug entrapment efficiency of approximately 77% could be achieved. Other techniques for improving drug loading and encapsulation of hydrophilic therapeutics include spray drying,[52,53] membrane emulsification,[54,55] and solid/oil/oil double emulsions.[56,57] Another emerging methodology is the conjugation of hydrophilic dendritic polymer segments to linear hydrophobic segments. The Watkins' research group has shown that this allows for both hydrophilic and hydrophobic drugs to be simultaneously loaded and delivered.[58]

2.2.2. Initial Burst Release

PLGA exhibits many characteristics that lend to its use in sustained-release formulations as detailed in earlier sections, including, but not limited to, excellent biocompatibility, degradability, and customizability. However, the problem of initial burst release has kept the biopolymer from realizing its full potential. Burst release is the uncontrolled kinetic phenomenon of significant quantities of the drug released from the particle not long after injection.[59] The result is lower availability of the drug for its intended use, and in some cases, local toxicity if the concentration becomes harmful to nearby tissues. Burst release is not a drawback unique to the mode of intravenous administration. The challenge of the intravenous carrier could be that the in vivo hemodynamics of blood flow creates shear, which leads to faster erosion and thus more rapid diffusion of the therapeutic from the polymer matrix. Depending on the blood vessel the particle is traversing at a given time, it will experience different shear rates and blood flow patterns (pulsatile, laminar). PLGA under shear may experience more detrimental burst release such that a significant amount of the drug may be released before reaching the target site. Other excellent reviews have acknowledged that the burst release problem is a major obstacle to translating what would otherwise be successful PLGA microparticle formulations.[60,61] Potential ways of mitigating burst release include alterations to drug–polymer interactions, surface permeability, copolymer sequence, and spatial arrangement of drugs in the polymer matrix. Yoo et al. is the most recent review to summarize these latest techniques in detail;[62] nonetheless, the problem is not fully resolved.

3. Challenges Associated with PLGA Surface Coating—The Pros and Cons of PEGylation as the Stealth Gold Standard

Polyethylene glycol was the first surface coating used to design long-circulating PLGA-based particles and remains the most investigated compound to date.[63] Once PEG has been engineered to attach to the hydrophobic PLGA surface, a variety of favorable physicochemical characteristics can be observed: namely, near-neutral zeta potentials,[64] a dense hydrated brush layer forming the outer shell,[65,66] steric stabilization in biological media,[67,68] and improved entrapment of water-soluble substances.[69,70] Depending on factors, such as PEG corona thickness, brush density, and molecular weight, different stealth properties will be

observed.[14] There appears to be an optimum molecular weight range (between 2 and 5 kDa) for PEG chains to repel opsonic proteins. Above this threshold, a significant reduction in opsonization will not occur.[14,68] Stealth performance also varies amongst the two modes of attaching PEG to PLGA (i.e., covalently bound or physically adsorbed).

3.1. Physically Adsorbed PEG Coatings

PEG is often physically attached to PLGA particles in the form of an emulsifier or surfactant coating. Many PLGA particles are fabricated using oil-in-water emulsion techniques. The PEG-based surfactant can be incorporated into the water or oil phase to make for a facile, one-step attachment to the PLGA core dissolved in the oily matrix. While there have been concerns surrounding the potential for gradual desorption of the PEG-based surfactant layers during in vitro and in vivo experimentation,[71–73] reports attesting to their stealth utility persists.

Chu et al. explored the physisorption route to fabricate sub-50 nm PLGA particles.[74] The oily emulsifier of interest was a compound known as poly(ethylene glycol)-distearyl phosphoethanolamine (PEGPE). The authors investigated different mass ratios of PEGPE to PLGA to optimize particle stability and reduce the tendency to aggregate. At a 3:1 ratio, the particles remained well-dispersed with no observable size changes after incubation in 10% bovine serum albumin (BSA) solution compared to its bare counterpart. The PEG coating also reduced burst release of the encapsulated drug, doxorubicin, and enhanced the passive cellular uptake by HeLa cells. Another study by Semete et al. investigated PEG and its surfactant derivative (Pluronics F127) to determine their influence on the biodistribution of PLGA nanoparticles post-oral administration.[75] The in vitro protein binding studies conducted therein revealed that at high plasma protein concentrations (~40%), the PEG-coated nanoparticles exhibited a reduction in protein binding while the use of a Pluronics coating experienced an increased protein binding. The biodistribution studies indicated that all particle types were present in all tissues one week after oral administration. However, the plasma concentration of stealth-coated particles was higher than that of bare particles. This latter observation proved that increased residence times could also be achieved when PEG is introduced into oral formulations. Because the stealth particles were detected in the liver, lung, and kidney at higher percentages than uncoated particles, the authors theorized macrophage uptake of the particles must still occur to some extent.

In another study, Godara et al. fabricated paclitaxel-loaded polymer-lipid NPs via a one-step nanoprecipitation approach using a variety of different surfactants.[76] In this study, the Pluronics (PF-68) surfactant was not as successful as the others (polyvinyl alcohol (PVA), tocopheryl polyethylene glycol succinate (TPGS), and human serum albumin (HSA)), by virtue of its quicker blood clearance times and relatively higher hemolytic activity. Another report by Ashour et al. attests to the benefits of physically incorporating PEG in PLGA nanoparticles to improve the entrapment efficiencies and drug loading of anticancer drug, 5-fluorouracil (5-FU).[77] Vij et al. showed that PLGA particles emulsified with a PEGylated phospholipid and loaded with a cystic fibrosis transmembrane conductance regulator (CFTR) could sustain accumulation in the lung tissue and reduce the presence of inflammatory macrophages and neutrophils.[78] This served as an indication of its ability to encapsulate the drug for an extended time while avoiding the MPS system. However, as this study lacked a bare particle control for adequate comparison, it is unclear how much this effect can be owed to the PEG coating rather than the PLGA capsule.

3.2. Covalent PEG Coatings

Covalent PEG coatings are indeed more abundant in the literature, perhaps partly due to the confidence of researchers in their long-term stability and the versatility of attachment or grafting techniques that have been developed for synthesizing amphiphilic PEG-PLGA conjugates.

Diblock copolymers of PEG and PLGA are especially prominent. Gref et al. were among the first to demonstrate a covalent attachment technique, reporting that nanospheres prepared from amphiphilic diblock copolymers of PLGA and PEG showed increased blood circulation times and reduced liver accumulation in mice.[79] Contrary to current consensus, these findings were linked to the molecular weight (MW) of the PEG component, with the authors hypothesizing that higher MW hydrophilic PEG blocks create a more effective steric barrier to opsonin adsorption. Ahmed et al. used factorial design to optimize the formulation of self-assembled nanoparticles based on PEG-PLGA for augmented vinpocetine brain delivery.[80] The vinpocetine plasma concentration-time curve and the brain concentrations post-intraperitoneal injection of the particles were analogous to those of the commercial oral tablets. Doxorubicin-loaded PEG-b-PLGA nanopolymersomes were also competitive to free drug solution in a study by Alibolandi et al.[81] The 140 nm vesicles with a therapeutic encapsulation efficiency of over 90% exhibited lower accumulation in the BALB/c mice lung and liver and significantly higher plasma concentrations than free Dox. Unfortunately, as both studies mentioned above did not include bare particle controls for assessment, it is difficult to know what degree to credit the PEG coating rather than the PLGA capsule for the positive performance.

Ferenz et al. probed the circulation and protein adsorption behavior of PEGylated PLGA microparticles with the specific intent to demonstrate their utility as suitable IV injectable carriers.[82] The circulation half-life of the particles in male Wistar rats was approximately 1 hour. The authors rationalized that the circulation time would be even longer when extrapolated to humans due to previous literature indicating that rats have a more robust RES.[83] Interestingly, the protein adsorption data within this publication did not corroborate the consensus surrounding PEG being a low-fouling coating. Instead, equivalent amounts of albumin (dysopsonin) and immunoglobulin G (opsonin) were adsorbed on the particle surface whether or not PEG was included in the formulation. Agnoletti et al. designed PLGA-PEG microparticles to passively target bacterial infections in the lungs using levofloxacin as the pharmaceutical cargo.[84] The passive targeting strategy involved intravenous injection of microparticles slightly larger than the lung capillary diameter. Hence, the particles are entrapped in the capillary vessels after entering pulmonary circulation. The authors fabricated monodisperse

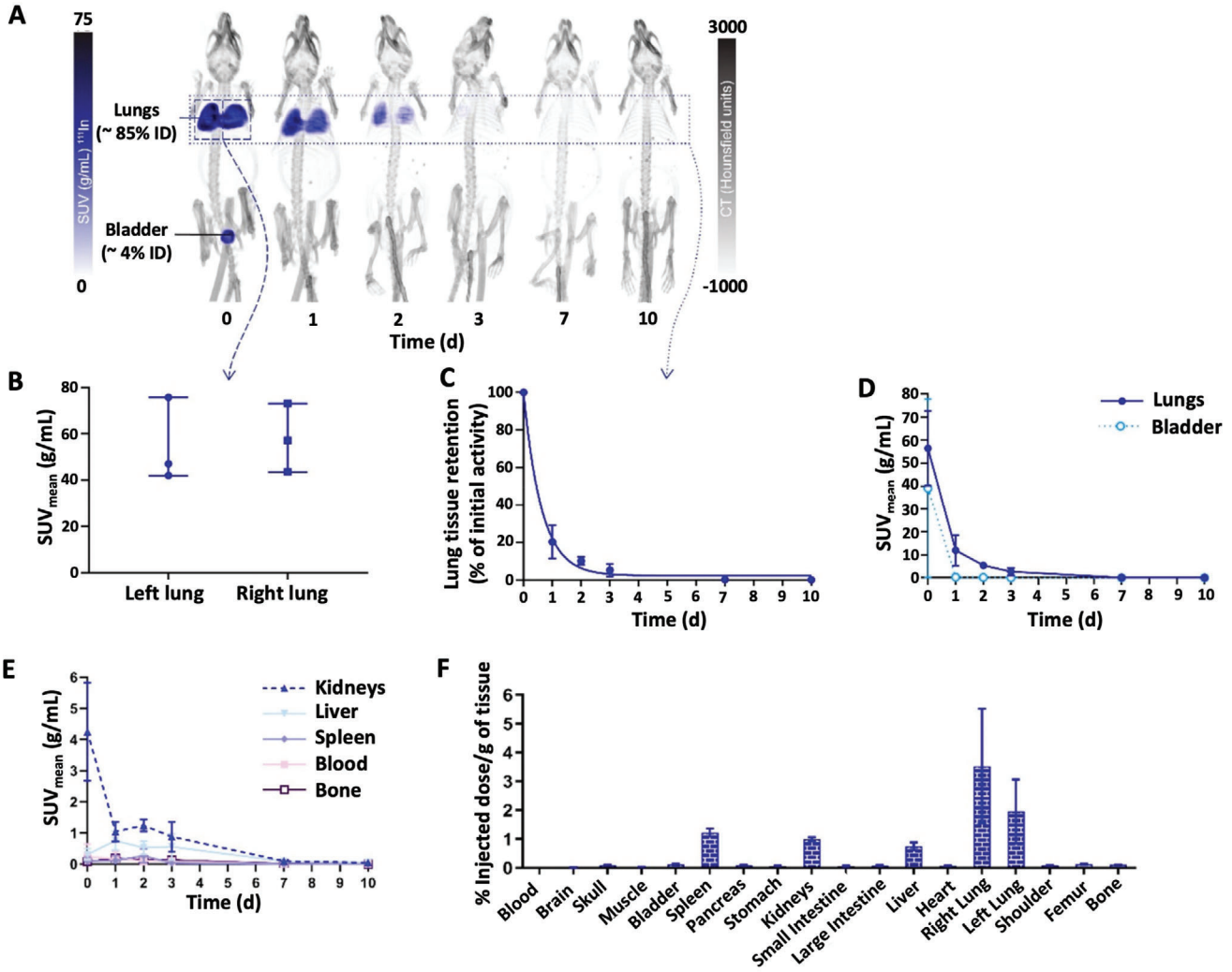

Figure 5. Pharmacokinetics and biodistribution of radiolabeled PEG- PLGA microspheres. A) Representative maximum intensity projection SPECT/CT overlay images (dorsal view) of healthy C57BL/6 mice showing the in vivo distribution of PEG-PLGA MS after IV tail injection over time. The radioactivity is shown in blue. B) Left and right lung distribution of PEG-PLGA MS immediately after IV injection, calculated from the SPECT images ($N = 3$), in standardized uptake value (SUV) (g mL^{-1}) (mean ± SD). C) Lung tissue retention of PEG-PLGA MS. D,E) Organ SUV in g mL^{-1} (mean ± SD) of the PEG-PLGA PLGA MS over 10 days, calculated from the SPECT images ($N = 3$). F) Biodistribution (mean ± SD, $N = 3$) of PEG-PLGA MS on day 10 after injection. Tissue uptake is expressed as % of injected dose (ID) per g of tissue. Adapted with permission.[84] Copyright 2020, American Chemical Society.

spheres with a mean diameter of 12 µm with a 50% weight composition of PLGA polymer and a 50% composition of PLGA-PEG-NH_2. Particles were radiolabeled with ^{111}indium and demonstrated preferential accumulation in the lung space with limited biodistribution in the other organs of the RES (see **Figure 5**). Many studies have explored the use of PEG-PLGA diblock or triblock self-assembled particles in intravenous cancer applications.[85–90] Most of these works investigate antitumor efficacy, drug-release kinetics, as well as blood circulation times and tissue biodistribution across mice models. The overarching trend in the results is that the PEG outer shell increases hydrophilic drug entrapment, sustains drug release kinetics, and substantially increases the half-life of the therapeutic.

As more publications of this nature continue to emerge, the question again arises as to why there exists such a large disconnect between (PEGylated) PLGA's performance in mice and its absence in the clinic. It is difficult to pinpoint precisely where the issue lies or which compounding factors are responsible. In the next section, we delve into some of the weaknesses of PEG and potential reasons why its success in mice has not translated over to humans.

3.3. The Disadvantages of PEGylation

Despite its immeasurable success and emergence as the "first of its kind" stealth technology, the PEGylation strategy still has known disadvantages. The accelerated blood clearance (ABC) phenomenon is perhaps the most widely recognized negative side effect of repeated dosage of PEGylated therapeutics. The

ABC is an unexpected immunogenic response that involves two preceding stages: anti-PEG antibodies induction and complement activation.[18] Many studies have implicated immunoglobulin M (IgM) as the major antibody produced by the spleen after the initial administration of PEGylated particles. IgM is not considered a direct opsonin that can signal phagocytosis alone since phagocytic cells do not possess an Fc receptor for IgM.[91] Instead, IgM acts as an indirect opsonin by activating the complement system. Complement factors, such as C3b, can then promote phagocytosis by Kupffer cells in the liver, which bear complement receptors. Unfortunately, this complement activation is also related to the cases of hypersensitivity (infusion) reactions that have been associated with PEGylated therapies.[92] Research has implied that a possible explanation of the ABC phenomenon's proclivity may be the growing prevalence of PEG in consumer products (e.g., shampoos, soaps, skin creams, and toothpaste).[93] Indeed, anti-PEG antibodies were detected in 72% of the population who had not received treatment with PEGylated therapeutics.[94] As organisms that do not contact PEG products at the same frequency, mice may tolerate the compound with greater ease than humans.

Besides ABC, PEG is characterized by a few more drawbacks that have fueled the transition to exploring stealth alternatives. Vacuolation of macrophages, epithelial tissues, and organs is one such side effect observed upon many dosages of parenterally administered high molecular weight PEG (> 10 kDa).[95] Although the formation of these membrane-bound organelles does not always carry toxicological significance, some studies have indicated potentially adverse effects due to irreversible changes in tissue architecture.[96] Additional weaknesses of PEG include its non-biodegradability and renal toxicity at high dosages.[97] Moreover, we recently showed that PEGylation of polymeric particles does not protect against phagocytosis in human blood.[98] Specifically, PEGylated particles were preferentially phagocytosed by human neutrophils over bare particles despite grafting densities previously shown to reduce phagocytosis in mouse blood and standard phagocytic cell lines. This observation is critical for drug carrier formulations since neutrophils make up 60–70% of white blood cells (WBCs) in human blood (unlike in mice where they make up only 20–30%);[99] thus, they are the primary phagocytes encountered by IV-injected particles in humans. PEGylated particles preferentially acquire complement proteins in human plasma, not prominent on bare particles, resulting in their preferential uptake by neutrophils in human blood. The complement level in the plasma in rodents has been reported to be low compared to humans,[100] explaining why PEGylation works for reducing phagocytosis in mice.[98] Additionally, most phagocytosis assays are performed with cell lines suspended in simple animal sera. The capability of PEG under these conditions does not directly extrapolate to humans. The bottom line is that blood is a tissue with distinctly different properties between humans and rodents. Thus, there is a need for new approaches to eliminate or positively alter plasma protein corona formation on polymeric carriers relative to human blood.

4. PEG Alternatives for PLGA Polymer Carriers

This section highlights some relatively underexplored coatings for designing long-circulating PLGA nanocarriers. A summary of the pros and cons of each class, along with PEG, for intravenous drug delivery is given in **Table 1** below.

4.1. Zwitteration

Poly-zwitterions remain the next most well-studied, second to PEG, non-fouling surface coatings used for biomedical applications. Zwitterionic compounds comprise molecules containing positive and negative charges bound covalently (see **Figure 6**).[101] The presence of these opposite charges in close proximity give rise to ionic solvation and result in a neutrally charged layer atop the substrate.[102] Water molecules align along the zwitterionic dipole moment, creating a tightly bound hydration layer. This hydration layer serves as a physical and energetic barrier to protein adsorption. The consensus is that this hydration layer is stronger than that of PEG. The most used zwitterions include poly(carboxybetaine), poly(sulfobetaine), and poly(phosphorylcholine). Current literature surrounding zwitteration in particulate drug delivery surrounds mostly substrates besides PLGA, including gold nanoparticles, silica, and quantum dots.[103] The few publications that outline zwitterion-coated PLGA's stealth capabilities are presented here.

Cao et al. were the first to covalently conjugate carboxybetaine on PLGA nanoparticles.[104] The sharp differences in polarity between super hydrophilic polyzwitterions, such as carboxybetaine, and the hydrophobic PLGA moieties make it challenging to find a common solvent for both reagents during synthesis. The authors overcame this barrier by designing a carboxybetaine monomer containing a *tert*-butyl ester group with good solubility in most organic solvents. Via atom transfer radical polymerization (ATRP), a block copolymer of PLGA and poly(carboxybetaine) (PLGA-PCB) was achieved. The PLGA-PCB nanoparticles fabricated from this polymer had a zeta potential (ZP) of −43.5 mV, while the bare particle control was more negative at −68.1 mV. Stability studies of PLGA-PCB NPs showed that these particles maintain their original size over five days in both 10 wt % BSA and 100% fetal bovine serum (FBS). In contrast, bare PLGA nanoparticles aggregated within the first three hours of immersion in these media. However, the study did not investigate the protein adsorption patterns of the PLGA-PCB particles in human plasma nor compare the particles to a PEGylated standard. Instead, superior stealth performance over PEGylated PLGA nanoparticles was assumed based on prior publications comparing the nature of the two coatings. In another study, Park et al. demonstrated that surface modification with polycarboxybetaine or PEG inhibited macrophage uptake of PLGA particles to similar extents.[105]

Zwitterionic phosphorylcholine-coated PLGA particles are more prevalent in the literature than their carboxybetaine counterparts. Phosphorylcholine (PC) is the hydrophilic group of the phospholipid, phosphatidylcholine, in the outer membrane of cells.[106] Thus, coating hydrophobic PLGA or PLA particles with this moiety confers a biomimetic nature. In designing lipid nanoparticles (non-PLGA) for tissue-specific mRNA delivery, Cheng et al. noted that increasing concentrations of zwitterionic phosphorylcholine lipids, DSPC and DOCPe, improved nanoparticle specificity to the spleen, diverting them from the liver.[107] The authors hypothesized that the altered biodistribu-

Table 1. Summary of the strengths and weaknesses of both PEG and its up-and-coming alternatives for intravenous drug delivery.

	Advantages	Disadvantages
PEG	Enhances ability of nanocarriers to cross blood-brain barrier, flexible, hydrophilic, soluble in a wide range of organic solvents, steric stabilizer, long clinical history	Hypersensitivity reactions, anti-PEG antibodies, non-biodegradable, causes enlargement of particle size, desorption of PEG layer over time, steric hindrance leading to reduced cellular uptake
Polyzwitterions	Super-hydrating, biomimetic, net neutral charge, high tunability (in the case of polycarboxybetaine), offers protein /peptide stability	Complex synthesis requirements, insoluble in organic solvents without additional modification, competitive self-association in the case of solfobetaine
Serum Albumin	Intrinsic biocompatibility, dysopsonin, abundant in human and bovine hosts, ease of purification, functionalizable	Possible immunogenicity, batch to batch variation, organic solvents can compromise integrity during fabrication
Chitosan	Hemostatic behavior, can be stimuli responsive to environmental pH, mucoadhesive, biodegradable, physioadsorption as a facile coating strategy, most abundant protein in the human bloodstream	Positive charge may lead to unwanted passive cellular uptake, slight hemolytic activity, questionable stealth character for some application
Red Blood Cell Membrane	120-day life span, intrinsic biocompatibility and immunity, low aggregation, long-term in vitro storage	Blood type matching an added complexity for treatment, expensive, regulatory red tape before achieving clinical translation, batch to batch variation, maintaining quality control, difficult to scale
White Blood Cell Membrane	13–20-day lifespan, built-in self-recognition mechanisms, receptors for disease sites, endothelial adhesion, and response to biological inflammation	Still in benchtop stages of development, heterogeneity in activation states of WBCs, low proportion of blood

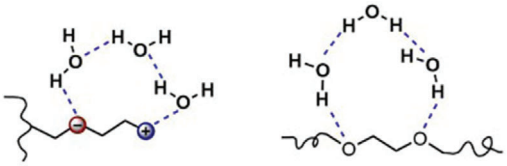

Figure 6. Different mechanisms of hydration: ionic solvation induced by zwitterions (left) versus hydrogen bonding on ethylene glycol units (right).

tion was due to a different protein corona and ionization constants conferred to the nanoparticles from the zwitterionic component. Bao et al. designed phosphorylcholine-based stabilizers that could be used in the water phase to coat PLA nanoparticles during the nanoprecipitation fabrication process.[108] Compared to the conventional surfactant, polyvinyl alcohol, the zwitterion-coated particles reduced phagocytic uptake by mouse peritoneal macrophages by 67% over the bare control. The cross-linked version of the PC stabilizer reduced the zeta potential of the PLA nanoparticles in half (−15 mV compared to −30 mV from control). Konno et al. also described phosphorylcholine on PLA nanoparticles.[109] They noted that the amount of BSA adsorbed on the nanoparticles was significantly smaller than that on the conventional polystyrene nanoparticles. Hsiue et al. prepared block copolymers of PC and PLA via ATRP.[110] The nanoparticles fabricated via the dialysis method had near-neutral zeta potentials (−6 mV), indicating the hydrophilic PC groups were primarily oriented at the particle shell. The low cytotoxicity and favorable biocompatibility of the particles were evidenced through assays with human fibroblast cells. However, biodistribution, circulation time, and protein adsorption were not assessed directly. Long et al. characterized the in vivo performance of star-branched PLA-block-PC copolymer micelles compared to PEG-PLA micelles.[111] The plasma half-life of the therapeutic in the former was 1.48-fold higher than the latter (19.3 h vs 13 h). Although the biodistribution in the liver and lungs was similar, PLA-b-PC accumulated less in the spleen, and the relative accumulation in the mice tumor was 2.37-fold higher than the PEG standard. There was no statistical difference between the amount of BSA adsorbed on the two nanomicelles. However, there was approximately 50% more fibrinogen on PEG-PLA micelles than on zwitterated micelles.

Sulfobetaine (SB) coated PLGA or PLA particles remain a rarity in the literature despite the numerous benefits and, at times, superior protein reduction on SB surfaces relative to the other non-fouling approaches (Debayle et al.).[112] Sun et al. described a biodegradable nanostructure based on SB-functionalized to a PLA backbone.[113] The nanostructure and corresponding conjugate with paclitaxel exhibited well-suppressed non-specific interactions with fibrinogen and acid-sensitive sustained drug release. Tu et al. synthesized a PEG-SB-PLA polymer hybrid and investigated its antifouling properties by preparing the polymer as a film on glass substrate.[114] Although the polymer was not prepared in colloidal geometry commonly used in intravenous drug delivery applications, the antifouling properties and wettability point to its tremendous stealth potential. The results showed that the PEG-PLA-SB polymeric surface was highly resistant to non-specific adsorption of four kinds of proteins (lysozyme, BSA, chicken egg albumin, and fibronectin) compared with the native glass surface. Both contact angle measurement and attenuated total reflectance-Fourier transform infrared (ATR-FT-IR) analysis showed that the polymeric surface had enduring stability. The introduction of PEG and SB to the PLA backbone greatly improved the hydrophilicity of the polymeric surface. Interestingly, the study did not include a PEG standard, making it difficult to assess how much of the effects were the SB component individually.

4.2. Polysaccharide and Protein-Based Coatings

4.2.1. Chitosan

Researchers had long been assumed neutrally charged layers to be the best-case scenario for limiting plasma protein adsorption

and shielding particles from interactions with the various blood components.[32,115] Somewhat overlooked but promising is using positively charged moieties, namely chitosan, as will be discussed in this section. A positive surface charge facilitates the attachment of these coatings to the negatively charged PLGA via electrostatic interactions.[116] An optimal cationic nature may also help prolong blood circulation via increasing ionic interactions with erythrocytes.[117] Aoki and coworkers reported in studies with liposomes that a slight positive charge density on the liposomal surface of around +15 mV resulted in prolonged residency of the cationic liposomes in rat blood compared to their neutral counterparts.[118]

Chitosan is a linear polysaccharide derived from treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide.[119] As the pKa of amino groups on the chitosan is around 6.5, they tend to remain protonated at acidic and neutral pHs and thus can exhibit mucoadhesive capabilities. Chitosan also exhibits a high positive charge which enables the biopolymer to interact electrostatically with the negatively charged components of the cell membrane. Thus, chitosan's pH sensitivity and mucoadhesive nature make it attractive and is prevalent in anti-cancer applications. As a coating, it can remain protonated in the weakly acidic tumor microenvironment and thus attach a particle substrate to the anionic glycocalyx that surrounds several cellular membranes.

Although much of the literature surrounding chitosan-coated PLGA has focused solely on the cellular association and antitumor efficacy of the drug delivery vehicle, a few reports have explored its ability to reduce opsonization and phagocytic uptake. Amoozgar et al. conjugated low molecular weight chitosan (LMWC) [2–22 kDa] to PLGA nanoparticles and obtained particles with zeta potentials that were slightly negative at a physiological pH (highest reported negative ZP = −12 mV) and positively charged at a pH of 6.5 (highest reported positive ZP = +14.9 mV).[120] J774A.1 mouse macrophages were incubated with bare PLA NPs and PLGA-LMWC NPs. Confocal microscopy demonstrated that PLGA-LMWC NPs effectively avoided uptake by J774A.1 macrophages, whereas they readily took up bare PLGA-NPs. Notably, this result was obtained at pH 7.4, where both NPs were negatively charged; therefore, contribution of electrostatic interactions with macrophages to the cellular uptake was minimal for both NPs. Instead, the result can be attributed to the hydrophilicity of the chitosan layer. Protein adsorption to the PLGA-LMWC microparticles (MPs) was significantly lower than bare PLGA MPs but not to PLGA-PEG MPs, as evidenced by the micro-bicinchoninic acid (BCA) assay.

Lee et al. prepared chitosan-modified PLGA nanoparticles and investigated their lung targetability and protein adsorption across different media.[121] The nanoparticles formed micro-sized aggregates in the bloodstream, improving their targetability to the lungs when compared with bare nanoparticles. The chitosan-modified particles also demonstrated higher rat plasma protein adsorption than bare nanoparticles and higher adsorption of the dysopsonin and albumin when incubated in human serum albumin solution.

Ahmad et al. studied chitosan-coated PLGA nanoparticles encapsulating rasagiline (RSG), a drug for treating Parkinson's disease.[122] The goal was to target these particles across the blood-brain barrier and into the brain tissue and compare which

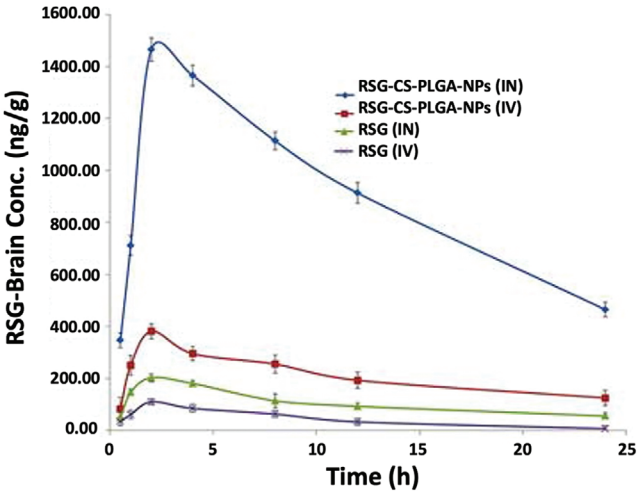

Figure 7. Pharmacokinetic profiles of rasagiline concentration in brain at different time intervals after administration of developed PLGA-NPs compared with pure rasagiline. RSG = rasagiline; RSG-CS-PLGA-NPs = chitosan coated rasagiline loaded PLGA nanoparticles; IV = intravenous; IN = intranasal. Reproduced with permission.[122] Copyright 2017, Taylor & Francis.

mode of administration (intranasal vs intravenous) produced better therapeutic efficacy. The mucoadhesive chitosan coating was used in this work to improve permeation across the blood-brain barrier. Some results from this study are characterized in **Figure 7** below: Although the authors found that the IV-injected chitosan particles helped enhance RSG circulation to the Wistar rat brain at all time points over a 24-hour study, intranasal delivery resulted in significantly better bioavailability. The more direct nose to brain route presented fewer biological barriers than the blood to brain pathway.

Chung et al. developed chitosan-Pluronic conjugated nanoparticles that were stable in full serum conditions and measured their biodistribution in tumor-bearing athymic mice.[123] The accumulation of chitosan-Pluronic PLGA NP in the liver was observed to be lower than those of other particle systems but was not significantly different ($p > 0.05$, $n = 3$). Yang et al. also observed the biodistribution of chitosan-modified PLGA nanoparticles compared to a bare control.[124] Although chitosan modification resulted in a dramatic increase in the distribution index of the drug to the lung compared to bare particles, distribution indexes decreased or remained constant for the other tissues or organs. The authors could not conclusively determine whether this chitosan coating mediated the uptake of nanoparticles by the RES by impeding opsonization. Interestingly, Durán et al. reported that chitosan-coated PLGA nanoparticles were preferentially ingested by primary human-antigen presenting cells, such as monocyte-derived dendritic cells.[125] This finding appears to contradict the characterization of chitosan as a stealth coating.

Other reports uphold the reputation of chitosan as a low-fouling, long-circulation conferring surface layer. In one study, Ishak et al. showed improved biodistribution of chitosan-PLGA compared to both a bare and polysorbate control.[126] These authors developed chitosan-coated PLGA NPs with greater surface hydrophilicity than the PEG and Pluronic-coated controls,

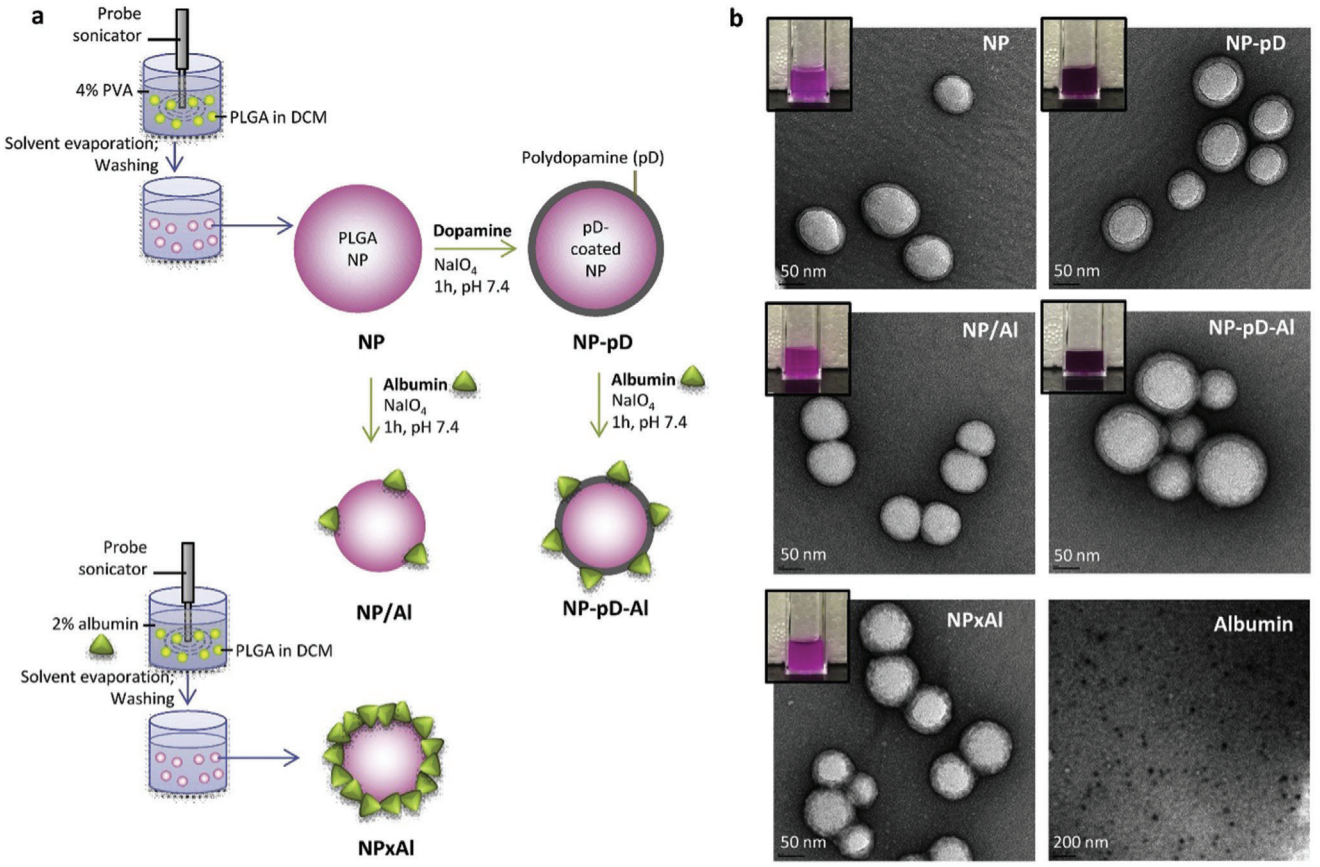

Figure 8. a) Different methods to conjugate albumin to PLGA NPs. b) Transmission electron microscopy (TEM) images of rhodamine-labeled NP, NP-pD (polydopamine conjugated nanoparticles), NP/Al (physically adsorbed albumin nanoparticles), NPxAl (surface embedded albumin nanoparticles), NP-pD-Al (albumin-polydopamine conjugated nanoparticles) and albumin, negatively stained with 2% uranyl acetate. Dark purple color of NP-pD and NP-pD-Al suspensions (inset) indicate the presence of polymerized dopamine (pD). Reproduced with permission.[135] Copyright 2018, Elsevier.

as measured by the adsorption constants in a Rose Bengal adsorption assay. The relative bioavailability of nanoparticles coated with chitosan to uncoated ones was computed as 0.26, 0.24, and 0.18, respectively, in liver, spleen, and kidney, respectively. Voon et al. designed a chitosan PLGA-photosensitizer nanoparticle construct that demonstrated decreased serum adsorption and macrophage uptake compared to bare controls.[127] The low molecular weight chitosan coating (25 kDa) enabled the particles to avoid the organs of the RES and exhibit higher accumulation at the breast cancer tumor site.

The discrepancy in chitosan's performance as a stealth coating amongst the different publications can be attributed to various factors. For example, chitosan in a lower molecular weight range (<25 kDa) tended to result in particles with more pronounced stealth outcomes than the study controls. Other factors that varied between studies included the method of surface modification (covalent vs physically adsorbed) and coating density.

4.2.2. Albumin

Serum albumin is the most abundant plasma protein across the mammalian species.[128,129] With a plasma half-life of over 15 days, it has been used to extend the circulation time of a wide variety of drug delivery vehicles, including liposomes,[130] polystyrene particles,[131] and silica particles.[132] As a globular, water-soluble, un-glycosylated serum protein with a molecular weight of about 65 kDa, albumin is utilized to transport both endogenous and exogenous compounds. Other functions include regulating colloidal osmotic pressure in the bloodstream. Unlike several other serum proteins, albumin is considered a dysopsonin; hence, a preformed albumin corona reduces opsonic serum protein adsorption and thus recognition by the RES.[131,133,134] Sobczynski et al. demonstrated that pre-coating PLGA nanoparticles with albumin helped improve their vascular targeting and adhesion across some donor blood in flow channels in vitro.[46] The reports of albumin-coated PLGA particles described herein have demonstrated much promise.

Hyun and coworkers studied three different surface modification strategies for albumin-modified PLGA and noted that the conformation of albumin elicited by each method resulted in different therapeutic outcomes.[135] See **Figure 8** below for a visual of the experimental. The authors determined that albumin performed best as a coating when it retained its native conformation—an outcome that was not possible when the protein was interfacially embedded at the particle surface.

The physio-adsorption strategy resulted in albumin being too weakly bound to the particle surface to make any significant dif-

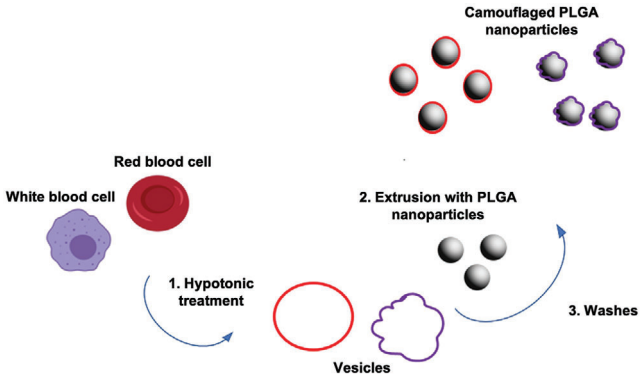

Figure 9. An Outline for the Steps Towards Coating PLGA Nanoparticles with Red Blood Cell Membranes and White Blood Cell Membranes.

ference in the experiments In contrast, the native protein conformation was well preserved during surface modification when the albumin conjugation was mediated with a polydopamine layer. The use of these particles led to more favorable experimental conclusions, including the least amount of mice serum adsorption after 2-h incubation in vitro, greater accumulation at the tumor site, and relatively low macrophage uptake. The interaction of albumin-polydopamine conjugated PLGA nanoparticles with J774A.1 macrophages matched that of its PEGylated counterpart. Manoochehri et al. and Esfandyari-Manesh and co-workers both explored human serum albumin coated PLGA for anticancer delivery of docetaxel and paclitaxel, respectively.[136,137] Both studies demonstrated cytotoxicity against tumor cells and sustained drug release over multiple weeks. Kesharwani et al. designed cationic BSA modified PLGA nanoparticles with markedly improved bioavailability and extended retention in systemic circulation post IV administration.[138] To summarize, modifying the hydrophobic particle surface with a preformed albumin corona presents a versatile strategy for reducing plasma protein adsorption, complement activation, and lengthening blood circulation time.

4.3. Cell-Mimetic Coatings

Within the last decades, researchers have begun to explore coatings that mimic components of the bloodstream in order to camouflage PLGA particles from circulating immune cells. In particular, red blood cell membranes and white blood cell membranes have been of interest and will be discussed in this section. See **Figure 9** for a brief visual representation of the cell membrane coating process.

4.3.1. Erythrocyte Membranes

Red blood cell membranes are a creative option due to their inherent ability to access all areas of the body, long circulation time, and well understood physiological behavior.[139] Erythrocytes originate in stem cells of the bone marrow and, upon maturation, circulate in the bloodstream for 3–4 months before becoming senescent and being cleared by macrophages. The low immunogenicity of red blood cells is partly due to the presence of the CD47 antigen on the cell surface. CD47 functions as a "marker of self," indicating that the cell belongs to the host and preventing its phagocytic uptake.[140,141] Close to half of the blood's volume is erythrocytes, so an abundant stock is available for isolation.[142] These unique characteristics have resulted in many publications elucidating the stealth and therapeutic capabilities of PLGA particles cloaked by red blood cell membranes.

Hu et al. were the first to describe PLGA nanoparticles encapsulated in red blood cell (RBC) vesicles.[143] The researchers utilized mechanical extrusion techniques to capture sub-100 nm PLGA particles into RBC vesicles without compromising the integrity and protein composition of the lipid bilayer membrane. Mice injection studies demonstrated that the erythrocyte membrane-camouflaged polymeric nanoparticles had an extended residence time over PEGylated controls. Biodistribution studies pointed to significant particle retention in mice blood 3 days post-tail vein injection. The data also suggested that the RBC-membrane-coated nanoparticles possessed comparable serum stability to PEG-functionalized lipid-polymer hybrid nanoparticles.

Aryal et al. designed erythrocyte cloaked PLGA nanoparticles loaded with doxorubicin via two different loading strategies, physical encapsulation, and chemical conjugation.[144] The chemical conjugation strategy led to a more sustained drug release profile due to the red blood cell membrane acting as a hindrance, delaying the outward diffusion of doxorubicin molecules. The cloaked PLGA nanoparticles were also more effective at killing acute myeloid leukemia cell line, Kasumi-1, than free doxorubicin.

No matter the pharmaceutical entrapped (e.g., resveratrol,[145] tetrandrine,[146] rapamycin,[147] curcumin,[148] or tirapazamine[148]), the data consistently points to beneficial stealth consequences of the drug delivery systems under inspection. Besides lengthy circulation times, carrier stability, and reduced liver accumulation, the degree of RBC membrane mimicry achieved has been quite precise. In nearly all publications, zeta potentials were not neutral (between −10 and +10 mV) but rather in the −20 to −30 mV range, which matched that of the red blood cell membrane vesicles. SDS-PAGE experiments repeatedly revealed that the protein expression pattern of the cloaked PLGA matches that of the membrane. CD47 expression was also similar, indicating the efficiency of the vesicle extrusion method to shield nanoparticles completely.[149] Few publications directly explored phagocytosis, but Ben-Akiva et al. and Wang et al. reported a reduction in RAW264.7 macrophage uptake for vesicle modified particles versus the bare control.[147,150]

While a promising and innovative top-down approach to camouflaging particulate drug carriers, the growth of this platform is dependent on several factors. The integrity of the membrane-derived vesicles must be preserved throughout both the extrusion and storage processes. Contamination or lesions that arise during latency could potentially lead to increase in mortality and unfavorable immunoregulatory responses.[151] Careful attention must also be given to the coupling of pharmaceuticals and ligands on RBC membrane surfaces as excess amounts will impact their elasticity and deformability.[152,153] This would increase risk of complement activation, leading to premature clearance from the bloodstream. Human RBC membranes also carry inherent

immunogenicity to the patient if they are not matched with that patient's blood type, similar to blood transfusion.[154] These challenges must be addressed before translation to a clinical setting, and further optimization is necessary.

4.3.2. Leukocyte Membranes

A handful of researchers have achieved coating of leukocyte vesicles on PLGA nanoparticles, and while the potential for stealth capability is there, most applications have surrounded anticancer and vaccine therapies. Macrophages, in particular, have long lifespans on the order of months or years.[155] They are considered professional phagocytes which respond to chronic inflammation and tissue damage. Upon activation, they are recruited to ingest and digest harmful invaders at the site of infection.[156] Similar to red blood cells, macrophages undergo a hypotonic lysis process to release cellular contents and acquire the outer membrane. Macrophage membrane camouflaged PLGA particles can then be obtained via extrusion or ultrasonication. Zhang et al. designed polyethyleneimine-modified macrophage cell membrane-coated PLGA nanoparticles containing model antigen ovalbumin and immunostimulant polysaccharides.[157] While these particles effectively attained macrophage specificity and prolonged antigen persistence, other experiments such as circulation half-life and serum protein adsorption were not conducted. The polyethyleneimine modification was used to attain high antigen loading capacity and enhanced uptake by negatively charged macrophages, a goal that is not consistent with stealth drug delivery.

In another study, Gong et al. described a hybrid membrane coating one PLGA, consisting of RAW264.7 macrophage membrane and 4T1 breast cancer cell membrane.[158] The PLGA core encapsulated doxorubicin. The hybrid structure was intended to simultaneously improve accumulation at sites of inflammation, target lung metastasis, and enhance tumor targeting efficiency. The system effectively achieved the antimetastatic effect, decreasing the number of metastatic nodules of breast cancer in the lung by 88.9%. Treatment with this system also prolonged the survival period of the organism over 20 days compared to the saline control. Notably, the macrophage-coated particles exhibited a significant decrease in biodistribution in the liver and increased distribution in the lungs, the target organ.

Other studies have evaluated the functionality of PLGA coated with membranes from primary leukocytes, i.e., neutrophils and monocytes. Neither neutrophils nor monocytes have particularly long lifespans in vivo;[159,160] however, the ability of their membranes to aid in escaping immune uptake and prolonging blood half-life has not yet been fully elucidated. Similarly, Kang et al. utilized neutrophil membrane-coated PLGA particles to treat circulating tumor cells.[161] Interestingly, plasma concentration data revealed that the neutrophil membrane camouflaged PLGA particles demonstrated a superior blood circulation profile compared with bare and PEGylated controls 48 h post-intravenous injection. Krishnamurthy et al. developed monocyte membrane-coated PLGA nanoparticles that demonstrate excellent serum stability for over 5 days.[162] These doxorubicin-loaded carriers were more successful in enhancing uptake by metastatic breast cancer cells and resulting in their cytotoxicity compared to non-coated nanoparticles. However, biodistribution, residence time, and protein adsorption experiments were not performed. The arena of leukocyte membrane coated PLGA holds untapped potential, and further investigation and fine-tuning are necessary to achieve desired stealth outcomes.

A summary of the different coating alternatives presented so far can be found in **Table 2** below.

4.4. Miscellaneous

There exist a few categories of coatings that have been explored minimally on PLGA-based colloidal carriers in the literature, which we describe in this section.

4.4.1. Poly(2-oxazolines)

Poly(2-oxazolines) (POx) comprise a versatile class of stealth compounds with alkyl side chains. With superior hydrophilicity and a more facile synthesis route via living cationic ring-opening polymerization techniques, POx is a fast-growing contender to PEGylation. The US FDA has approved using one member of the POx family, poly(2-ethyl-2-oxazoline), in food additives, attesting to its low toxicity and general biocompatibility. Extensive literature points to POxylation of biomacromolecules such as polystyrene,[163–165] albumin,[166] uricase,[166] liposomes,[167–169] and polycaprolactone.[170–172] In several reports, the antifouling properties of POx were useful in limiting protein adsorption,[173–175] extending particle circulation time, and reducing accumulation in organs of the RES system.[176–178] Bauer et al. determined that POx had mostly comparable cytotoxicity and hemocompatibility to PEG when controlling for molar mass and dosage.[179] The reports of POx on PLGA are sparse, and even fewer directly explore stealth behavior such as reduced phagocytic uptake and/or lengthy circulation time. In one study, Leiske et al. developed PLGA micro- and nanoparticles using POx-based surfactants. They hypothesized that the physicochemical properties conferred by POx would be useful for improving the particle's cell-specific targeting and cryoprotective abilities.[180]

Amphiphilic comb polymers containing poly(lactic acid) and poly (2-ethyl-2-oxazoline) were prepared by Yildirim et al.[181] These self-assembled in aqueous solution, forming spherical and worm-like micelles capable of entrapping drugs and exhibited relatively low lysosomal uptake. The first report of a poly(2-ethyl-2-oxazoline)-*co*-PLGA block copolymer was perhaps by Dirauf et al. and involved a one-to-one comparison to its analog, PEG-b-PLGA of similar composition and size.[182] The comparison largely surrounded chemical characterization and select colloidal properties such as zeta potential and hydrodynamic diameter. Overall, based on the parallel agreement of these parameters, the authors hypothesized that the POxylated PLGA micelles could be a suitable alternative to its PEGylated counterpart. Further biological assays were recommended to assess plasma protein binding, degradation, and drug encapsulation efficiency and release. In a different study, doxorubicin-loaded POx-*co*-PLA micelles with pH-sensitive release characteristics were prepared with near-neutral zeta potentials and good stability under phys-

Table 2. Summary of the composition, size, pharmacokinetic behavior, biodistribution, and excretion route for the stealth alternatives to PEG. Not intended to be exhaustive (PEGPE: 1,2-distearoyl-sn-glycero-3-phosphooethanolamine-*N*-[methoxy(polyethylene glycol)-2000], Administration route is oral).

Surface coating	Composition	MW [kDa]	Hydrodynamic Size	ZP [mV]	Residence Time or Half-Life	Pharmacokinetics	Biodistribution	Reference
PEG	PEGPE	2	< 50 nm	−19	—	—	—	Chu et al. (2011)[74]
	Pluronics F127	10	250–440 nm	30	2–4% of particles in plasma at day 7	—	liver, lungs, kidney	Semete et al. (2012)[75]a
	PEG	9			5–10% of particles in plasma at day 7			
	Pluronics F68	8.4	260 nm	−12	>500 ng mL^{-1} at 24 h	Maximum plasma concentration is 3600 ng mL^{-1}	—	Godara et al. (2000)[76]
	PEG	6	175–250 nm	−7 to −13	—	—	—	Ashour et al. (2019)[77]
	PEG PS341	—	121.1–136.5 nm	—	—	—	lungs	Vij et al. (2010)[78]
	PEG	5	—	—	—	—	liver	Gref et al. (1994)[79]
		3.5	134 nm	—	2–5 µg mL^{-1} drug at 24 h	Serum stability over 6 months	liver, lungs, spleen	Alibolandi et al. (2015)[81]
		5	1.5 µm	—	Half-life ≈ 1h	—	—	Ferenz et al. (2013)[82]
		12	78–85 nm	−0.97	Half-life 13 h	Dil still present at 72 h	spleen, lungs, liver	Long et al. (2016)[111]
Zwitterions	Carboxybetaine	—	149 nm	−43.5	—	—	—	Cao et al. (2010)[104]
		9.4	50–1000 nm	0–5	—	—	—	Park et al. (2014)[105]
	Phosphorylcholine lipids (DSPC and DOCPe)	—	—		—	—	spleen, lungs, liver	Cheng et al. (2020)[107]
	Phosphorylcholine (MPC)	> 6.6	119–179 nm	<−30	—	—	—	Bao et al. (2014)[108]
		—	221 nm	−2.5	—	—	—	Konno et al. (2001)[109]
		6–8 (cutoff)	118–156 nm	−6	—	—	—	Hsiue et al. (2007)[110]
		4.72	64.5 nm	−4.3	Half-life 19.3 h	Dil present at 72 h	Liver, lungs, spleen	Long et al. (2016)[111]
	Sulfobetaine	—	19.3 nm	—	—	—	—	Sun et al. (2017)[113]
	PEG & sulfobetaine (MPEG-PSBMA)	10.4	—	—	—	—	—	Tu et al. (2013)[114]

(*Continued*)

Table 2. Continued.

Surface coating	Composition	MW [kDa]	Hydrodynamic Size	ZP [mV]	Residence Time or Half-Life	Pharmacokinetics	Biodistribution	Reference
Chitosan	Low MW Chitosan	2–22	175–480 nm	−12 to 15	—	—	—	Amoozgar et al. (2012)[120]
	Chitosan (CS05)	45–50	388 nm	12	Detected in human serum, rat plasma, and di-water at 24 h	—	Liver, spleen, kidney, heart, lungs	Lee et al. (2016)[121]
	Chitosan-Pluronic	10	100–150 nm	−50 to +38	—	Particles detected in tumor at 72 h	Liver, heart, lungs, kidney, spleen	Chung et al. (2010)[123]
	Chitosan	45–50	200–300 nm	8–32	—	Plasma concentration decreased over 12 h	Plasma, heart, liver, spleen, lungs, kidney	Yang et al. (2009)[124]
		—	150 nm	26	—	≈70% FA positive cells at 24 h		Duran et al. (2019)[125]
		—	319–393 nm	−18 to 10	Half-life 45.88 h	AUC (0→infinity) at 16.99 μg h mL^{-1}	Liver, kidney, speen	Ishak et al. (2013)[126]
	Low MW Chitosan	25	147 nm	—	—	—	Lymph nodes, spleen, liver	Voon et al. (2016)[127]
Serum Albumin	Bovine (BSA)	(BSA) 60–70	440 nm	—	—	—	—	Sobczynski et al. (2017)[46]
	Human (HSA)	(PLGA) 25–35	160–200 nm	−2 to −4	—	Low J774A.1 macrophage uptake	Liver, spleen, highest in tumor, low accumulation in kidneys and lungs	Hyun et al. (2018)[135]
		(PLGA) 48	204 nm	−6	—	—	—	Manoochehri et al. (2013)[137]
			190–220 nm	< −6	—	—	—	Esfandyari-Manesh et al. (2015)[136]
	Cationic Bovine (cBSA)	—	121 nm	−6	—	Coated NPs at 3X greater plasma concentration than bare: 0.68 versus 0.27 μg mL^{-1}	Liver, kidneys, spleen, highest in tumor, low accumulation in heart	Kesharwani et al. (2016)[138]

(*Continued*)

Table 2. Continued.

Surface coating	Composition	MW [kDa]	Hydrodynamic Size	ZP [mV]	Residence Time or Half-Life	Pharmacokinetics	Biodistribution	Reference
RBC Membrane	RBC ghosts from male imprinting control region (ICR) mice	—	85 nm	−10	Elimination half-life 39.6 h	Particles exhibit 29% and 16% blood retention at 24 and 48 h, respectively	Highest accumulation in blood and liver	Hu et al. (2011)[143]
	RBC ghost from male ICR mice	(PLGA) 10	90 nm	—	—	—	—	Aryal et al. (2013)[144]
	RBC from Sprague Dawley rats	(PLGA) 15–24	190 nm	−20	Half-life 10.34 versus 7.31 min for bare particles	Mean residence time 11.3 versus 8.7 min for bare particles		Li et al. (2019)[145]
		(PLGA) 30	169 nm	−17	Half-life 19.38 h (3 times free drug)	Mean residence time 27 versus 3 h for free drug		Que et al. (2019)[146]
	RBC from C57BL/6 mice	(PLGA) 90	97 nm	−29		31% and 17% retention in blood at 24 and 48 h, respectively	Highest accumulation in liver and kidneys	Wang et al. (2019)[147]
	RBC from humans		105 nm	−31				Bidkar et al. (2019)[148]
WBC membrane	Mouse peritoneal macrophages from female ICR mice	(PLGA) 18	156–176 nm	−27, +31				Zhang et al. (2020)[157]
	A fusion of RAW264.7 macrophage and 4T1 breast cancer cell	(PLGA) 35	142 nm	−31		Low pH responsive drug release; prolonged circulation		Gong et al. (2020)[158]
	Neutrophils from male ICR mice		100 nm	−35	Half-life 6.59 h, PEG-control 4.73 h, bare 0.77 h		Highest accumulation in liver and lungs (target)	Kang et al. (2017)[161]
	U937 monocytes		< 200 nm	−17		Serum stability over 120 h		Krishnamurthy et al. (2016)[162]

iological conditions, both of which are promising indicators for intravenous injection.[183]

4.4.2. Polyvinyl Alcohol

Polyvinyl alcohol (PVA) is a hydrophilic synthetic polymer consisting of vinyl acetate and vinyl alcohol repeat units. As the most commonly used emulsifier for fabricating PLGA particles by the nanoprecipitation and solvent evaporation method, PVA forms an interpenetrating network on the surface of colloidal carriers.[184,185] It imparts a level of hydrophilicity to the biodegradable polyester particles as residual amounts of the emulsifier remain after repeated washing and centrifugation cycles. PVA stands out amongst the several stealth coatings presented herein because of its prevalence as an intrinsic part of the formulation process. Although few appreciate PVA as a stealth coating, there exists literature that points to its steric stabilization effects. In one study, Sahoo et al.[186] demonstrated that as the amount of the PVA in the water phase increased from 0.5% to 5%, the zeta potential of the particles increased from approximately −15 to −7.5 mV. Particle hydrophobicity likewise exhibited an inverse relationship to the surfactant concentration. Nanoparticles with higher amounts of residual PVA also experienced a reduction in non-specific uptake and were less likely to be ingested by human arterial vascular smooth muscle cells. PVA has also been implicated alongside PEG in reducing adsorption of opsonins such as immunoglobulin G on PLGA microparticle surfaces.[80] A previous study by Torchè et al. suggested that residual PVA content on PLGA microspheres may be partly responsible for reducing particle phagocytosis by pig alveolar macrophages.[187] Although reports continue to attest to the hydrophilicity conferred to PLGA particles by polyvinyl alcohol,[188] few researchers utilize PVA at the high concentrations (5% or greater) required to observe these outcomes when fabricating PLGA-based colloidal carriers. Instead, the formulation parameter tends to serve the sole purpose of preventing coalescence of the oil phase droplets during fabrication. For this reason, there exist no studies of PVA covalently attached to PLGA, but a plethora of literature on the physisorbed PVA coating, the latter of which is less permanent. In another study,[189] PVA hydrogel-coated PLGA microspheres were utilized in a composite form by Gu et al. to suppress the foreign body response of glucose sensors. Although these spheres were too large for IV applications (≈34 μm in diameter) and the composite was implanted subcutaneously, the coating still served its intended non-fouling, resembling soft human tissue due to its high water content and elastic nature.[190] It helped maintain continuous release of the drug dexamethasone while extending the sensor lifetime by minimizing bioenvironmental interactions.[191] Despite all these promising results, more extensive investigation is needed to determine whether a polyvinyl alcohol surface coating can be competitive against PEG and alternative coatings.

4.4.3. Ionic Liquids

Ionic liquids (ILs) represent a new and exciting class of stealth coatings for PLGA particles. This coating was developed by Hamadani et al. within the last year.[192] Ionic liquids are salts with melting points below 100 °C.[193] The structure is composed solely of bulky cations and anions with an asymmetric arrangement. Ionic liquids have long been viewed as promising replacements for conventional organic solvents. They find use across several commercial applications outside of synthesis, from batteries to waste recycling[194] to tribology.[195] ILs can be synthesized from materials with high biocompatibility, including those already found within the human body, and ingredients approved by the FDA as medical, food, or cosmetic additives. One promising sub-class are ILs composed of choline carboxylic acids. Choline, a quaternary ammonium cation, is closely related to neurotransmitter alkyl choline, and many short-to-medium length carboxylic acids are used as flavorants. Hamadani et al. used this sub-class and investigated the role of the cation:anion ratio and the identity of the cation and anion. Choline 2-hexanoate (1:2) was the IL with the highest protein resistance. The superiority of IL-coated PLGA particles compared to bare and PEGylated PLGA controls was evidenced by their having the highest retention in BALB/c mice 24 h post-intravenous injection (≈35% of injected dose) and lowest levels of protein adsorption after incubation with mouse serum. The data suggested that the protein avoidant ionic liquid coating enabled the PLGA nanoparticles to attach to erythrocytes in the bloodstream, extending their circulation time in vivo. Biodistribution studies also demonstrated favorable outcomes with 50% of dosage remaining in the lungs and less than 5% in the liver. IL-PLGA spheres also did not activate interleukin-6, an inflammatory cytokine in mouse plasma, 24 h post-injection compared to the particle controls. The field of drug delivery would greatly benefit from future studies exploring ionic liquids on PLGA and other particle substrates.

5. Conclusion and Future Outlook

In this review, we explore the lag of PLGA-based colloidal carriers relative to their drug delivery contemporaries in the realm of clinically approved IV formulations, highlighting the gaps in research that may be preventing the clinical translation of injectable PLGA nanoparticles. We focus on the hydrophobic nature of PLGA particle surface and summarize PEG-based surface engineering strategies' dominance as the go-to approach for achieving low-fouling surfaces to aid PLGA's utility as a long-circulating drug system in intravenously formulations despite the many other potential alternatives that exist. We posit that this laser focus on PEG may be a critical piece hindering the progress of PLGA in intravenous drug delivery applications. Hence, we have presented several potential alternatives to PEG and the research gaps that may be limiting their clinical translation relative to PEG.

To assess the superiority of one coating over the other, the field would benefit from more studies comparing the alternative stealth coatings to not only a bare particle control but specifically to a PEGylated particle standard. Few studies, however, make direct comparisons to PEG or even to one another. The general utility of many of the coatings remains somewhat ambiguous because a minority of publications test protein adsorption in *human* plasma or examine uptake by primary human phagocytes, but rather in animal serum or diluted plasma. Many publications

also tend to stop short of the thorough in vivo animal characterization necessary to assess toxicity, longevity, and biodistribution definitively. While zeta potential measurements and protein adsorption assays provide hints to particles' performance once in dynamic circulation, several factors cannot be accounted for (e.g., shear rate, allergic responses, and blood pressure fluctuations) with in vitro work alone. We have also pointed out in published works that mice models may not be the ideal for representing particle-blood interactions in humans.[196] Rather, porcine models appeared to serve as a better preclinical model for predicting human in vivo functionality.

Polyzwitterions may be the runner-up to PEG since poly(carboxybetaines) and phosphorylcholines have been granted FDA approval on other particle systems besides PLGA. However, biomimetic coatings such as erythrocyte membranes have a long trail of success across animal models and show great potential. Predictions aside, it should be noted that the invention of long-circulating stealth particles is a rather complex undertaking that requires a detailed understanding of the intricate interactions between particles and their biological environments. The pathway from the benchtop to the clinic is never linear and is often muddled with seemingly contradictory findings and discrepancies between laboratory performance and clinical outcomes. At times, preconceived notions about what a viable stealth coating should look like causes researchers to overlook possible candidates (i.e., the theory that neutrally charged surfaces are always more repellant to opsonization or maximal reduction in protein adsorption is required for long circulation). Rather than designing extensively non-fouling coating, it may be beneficial to identify unique particle protein coronae that confer stealthiness and focus on surface coatings that help acquire these. Additionally, polycarbonate, polyphosphoesters, and polyvinylpyrrolidone represent stealth coatings tried on various substrates but not yet on PLGA. It must also be acknowledged that compared to PEG, most coatings are still in their infancy, particularly at the proof-of-concept stage. Later down the line, as the different compounds find their way into the clinic, they too may or may not encounter adverse antibody effects after repeated administration. In this case, it may be advantageous to seek out cell mimetic coatings based on primary human cell lines or other naturally derived substances as opposed to synthetic ones. There exist potential difficulties in translating cell-based coatings to humans—namely the need for high throughput methods for manufacturing cell membrane coatings on the gram scale, the need for aseptic and long term storage conditions to preserve batch purity, and the need for type matching between the donor and the source cell material.[139] Personalized medicine could play a role in overcoming this latter obstacle, but all challenges must be sufficiently addressed before large-scale human treatment can begin.

Overall, this review highlights the need to consider the clinical underperformance of PLGA. Are we missing out by failing to explore alternatives to PEGylation? How can we reconcile success in animal models with systematic failures clinically? The answer must lie in part at the particle-human body interface and is undoubtedly beautifully complex. We call on the drug delivery community to join us as we continue to rise to this challenge with an open mind.

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Conflict of Interest

The authors declare no conflict of interest.

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