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Poly(lactic-co-glycolic acid) nanoparticle fabrication, functionalization, and biological considerations for drug delivery

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ABSTRACT

Nanoparticles can be used for drug delivery and consist of many sizes and chemical compositions. They can accommodate a diverse population of drugs and can be made to target specific areas of the body. Fabrication methods generally follow either top-down or bottom-up manufacturing techniques, which have differing production controls, which determine nanoparticle characteristics including but not limited to size and encapsulation efficiency. Functionalizing these nanoparticles is done to add drugs, prevent aggregation, add positive charge, add targeting, etc. As the nanoparticles reach the target cells, cellular uptake occurs, drug is released, and the nanoparticle is broken down. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles have often been used for drug delivery applications as they have shown minimal toxicity, which has helped with US FDA approval. This review breaks down PLGA nanoparticle fabrication, functionalization, and biological considerations.

I. INTRODUCTION

Poly(lactic-co-glycolic acid) (PLGA) nanoparticles have been used as a vessel for drug delivery for some time. Some examples of PLGA nanoparticles are included in Table I. PLGA is an established US FDA approved polymer used in many nanoparticle applications due to its high biocompatibility and biodegradability.[1–3] Bare PLGA nanoparticles, however, will not do much inside of the body and so research has been done to functionalize these nanoparticles for various applications. With the newly targeted applications, researchers needed to consider new sizes, shapes, drugs, surface features, and biology, which are largely accompanied by new fabrication methods.

Two major methods exist to fabricate nanoparticles: emulsification-solvent evaporation and precipitation. Each has its advantages and disadvantages, with emulsification-solvent evaporation being able to be more easily scaled up where precipitation can achieve higher encapsulation efficiencies. Precipitation also includes the popular microfluidic methods that report fine-tuning of nanoparticle size, charge, and encapsulation efficiency. PLGA nanoparticles can be functionalized by choosing what drug is being loaded and by modifying the nanoparticle surface. After the nanoparticles are in the body, researchers need to consider passive targeting, the efficiency of cellular uptake, the release profile of any carried drugs, and the degradation/clearance/toxicity.

This review aims to provide an overview of PLGA nanoparticles starting with their fabrication, then how they are popularly functionalized, and finally what factors are often considered once the nanoparticles interact with the body. A researcher can use the contents of this review to jumpstart their design of a PLGA nanoparticle for a new application.

II. PLGA NP FABRICATION METHODS

A. Emulsification-solvent evaporation (top-down)

Top-down manufacturing is a process where an item is made by breaking down larger items like chopping wood. In terms of

TABLE I. List of US FDA approved drugs that contain PLGA. Note the approval years, some older and some newer.

Drug name	Active ingredient	Company	Application	Year approved
Lupron Depot	Leuprolide acetate	AbbVie Endocrine Inc	Advanced Prostatic cancer	1989
Sandostatin LAR	Octreotide acetate	Novartis	Acromegaly, carcinoid tumors, Vasoactive intestinal peptide Tumors	1998
Eligard	Leuprolide acetate	Tolmar Therapy	Advanced prostatic cancer	2002
Risperdal Consta	Risperidone	Janssen Pharms	Schizophrenia, bipolar disorder	2007
Bydureon	Exenatide synthetic	AstraZeneca AB	Type 2 diabetes Mellitus	2012

PLGA nanoparticle synthesis, top-down refers to the breaking down of a bulk precursor to nanoparticles using some mechanical method. As seen in Fig. 1, one common method is emulsification-solvent evaporation, which consists of applying high shear force (i.e., sonication/ultrasonication) to a mixture of a small amount of PLGA dissolved in water-immiscible organic solvent and a large amount of surfactant dissolved in water and then evaporating out the organic solvent.[4–10] Many examples of various single emulsion techniques have been explored with varying success.[5,8–14] The initial mixture does not form any nanoparticles since the organic solvent is not miscible in water, and so pockets of PLGA in organic solvent float around in the water, unable to form nanoparticles until agitated in some way. Using sonication as the example shear force, once the probe sonicator is introduced, the energy introduced forces the water-immiscible organic solvent to mix with the water, thus exposing PLGA to water. Since PLGA is hydrophobic, it will clump together in water and form nanoparticles. In this sense, the nanoparticles formed have no layer or bilayer and are, instead, simply a collection of PLGA polymers in a ball. The size of the nanoparticles that form initially is large but continues to decrease as the sonication continues. While sonication causes the initial mixing and nanoparticle formation, it also breaks up the nanoparticles after they have been formed to allow them to reform, typically at a smaller size. When taken to a limiting amount of time, nanoparticles will be broken down and reformed to the same size, based on the sonicator strength, and the sonication is complete. The removal of the organic solvent via evaporation reduces the nanoparticle size and is necessary afterward because the organic solvent is harmful to the nanoparticle actions and toxicity. PLGA nanoparticle properties can be detected in numerous ways including but not limited to electron microscopy, Dynamic light scattering (DLS), AFM, and fluorescence detection.[15]

Since this organic solvent is immiscible in water, some examples are triethylamine,[16] dichloromethane (DCM),[5–8,12–14,16–24] chloroform,[9,16,25,26] diethyl ether,[16] and ethyl acetate.[11,16] Many considerations come into play when determining which organic solvent is right, but sometimes, the most important parameter for fabrication is the length of the evaporation step. Diethyl ether has the shortest evaporation step and so it would be chosen if time was the largest factor.[27] The toxicity of the chosen organic solvent may also be a greater factor when going for US FDA approval as substances like chloroform are highly toxic and need to be 100% removed to be deemed safe and getting to 100% removal will inevitably increase either fabrication time or cost.

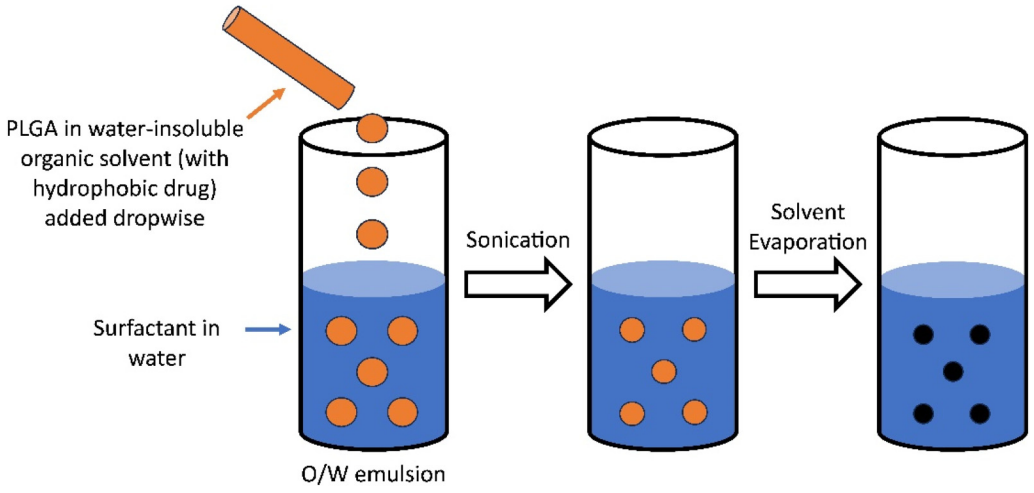

FIG. 1. Emulsification-solvent evaporation single emulsion process. Sonication used as an example method to apply high shear force.

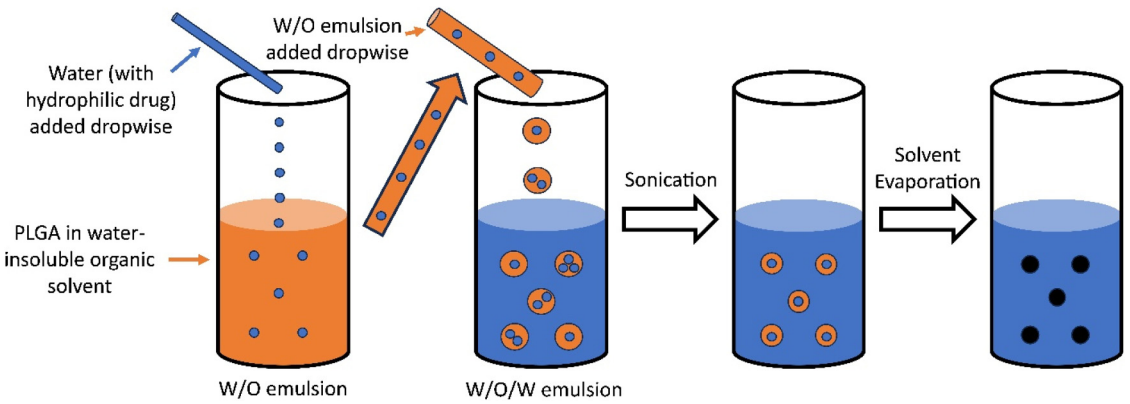

FIG. 2. Double emulsion process. Sonication again used as an example method to apply high shear force.

Described earlier was single emulsion, where hydrophobic drugs would be used, but double emulsion has also been demonstrated, which allows the use of hydrophilic drugs as shown in Fig. 2.[6,7,17–26,28] Double emulsion literally means two emulsions and consists of adding a small amount of water to the organic solvent before the organic solvent is added to the bulk water. Hydrophilic drugs are dissolved in the small amount of water added to the organic solvent. This is better than just adding hydrophilic drugs to the bulk water as the encapsulation efficiency is higher since more drug is near the PLGA when the nanoparticles are forming. If you add hydrophilic drugs to the bulk water, then there is a higher chance the drug never reaches any PLGA and, thus, is never encapsulated.

B. Precipitation (bottom-up)

Bottom-up manufacturing refers to the building up of a larger item from smaller pieces like building a house. For PLGA nanoparticle synthesis, bottom-up means forming nanoparticles from single polymer strands of PLGA. This has been done by adding an organic solvent to water just as before in a process called precipitation (nanoprecipitation for nanoparticle formation, except this organic solvent is miscible with water).[4,29–40] With light magnetic stirring, the two phases mix readily and form nanoparticles immediately.

The organic phase used here needs to be miscible in water, so some examples are acetone,[16,30,31,33,35,37,39,40] acetonitrile,[16] dimethyl sulfoxide (DMSO),[16] and tetrahydrofuran (THF).[16,32] Again, the choice would be based on several factors including evaporation time and toxicity.

To manufacture a significant amount of nanoparticles for clinical and commercial applications, the scale-up of the process is necessary. These methods need to overcome several obstacles including reproducibility, toxicity, and surface chemistry.[41,42] Existing solutions use membrane extrusion,[43,44] supercritical fluid technology,[45] and spinning disk processing[46] each with its own advantages and disadvantages.

An adaptation to the previously mentioned bulk methods is the use of microfluidics and hydrodynamic flow focusing (HFF).[47–52] In the HFF technique, the two phases are prepared the same but are then injected into a micro-/nano-scale polydimethylsiloxane (PDMS) device, which is manufactured by a combination of UV lithography and PDMS molding steps, where the two phases meet. In this type of device, the organic phase is pinched on both sides by the water phase. As soon as the phases meet, diffusion begins resulting in nanoparticle formation. To control the size and encapsulation efficiency, one can adjust several parameters including each phase's flow rate, the flow rate ratio, the channel length, all concentrations, and the materials are used. An additional material consideration with microfluidic methods is the interaction between PDMS and PLGA. Since normal PDMS is hydrophobic, it attracts PLGA and, by extension, the PLGA nanoparticles, leading to aggregation in the channels and eventually clogging. Methods have been developed to prevent this aggregation/clogging such as using oxygen plasma to oxidize the surface of the channels, which makes the surface hydrophilic and, thus, repels PLGA and the formed nanoparticles. Another challenge with microfluidics is the scale-up of the process as the working volume, though advantageous for size control and encapsulation efficiency, also limits the throughput.[53,54] For microfluidic devices, scale-up is necessary to increase the naturally low throughput and can be achieved by employing parallelization and switching from PDMS to another material as PDMS cannot handle the higher flow rates required for increased throughput.[55–57] Since many microfluidic scale-ups require making a more complex device, increases in the manufacturing cost and/or time can be expected.

After nanoparticles are produced by any method, they need to be stored properly to maintain their stability. The method (i.e., aqueous, freezing, and lyophilized storage) and type of cryoprotectant (i.e., sucrose, trehalose, or mannitol) greatly affect stability with notably the only US FDA approved COVID-19 mRNA vaccines, which are made from lipid nanoparticles, being stored in freezing conditions with sucrose.[4,58,59]

Both fabrication methods can benefit greatly from various PLGA nanoparticle manipulation techniques. Bulk methods tend to employ ultracentrifugation,[15,60,61] ultrafiltration,[15,62] size exclusion

chromatography,[15,63] precipitation,[15,64] or immunoaffinity techniques,[15,65] which are used to separate, purify, and concentrate the PLGA nanoparticles. Microfluidic methods can employ either active or passive techniques. Active techniques include acoustic,[15,66] electric,[15,67] magnetic,[15,68] and optical controls,[15,69] whereas passive techniques include inertial microfluidics,[15,70] deterministic lateral displacement,[15,71] microfluidic filtration,[15,72] pinched flow fractionation,[15,73] and viscoelastic microfluidics.[15,74] Both active and passive techniques aim to control particles with active methods tending to be more precise but having lower throughput and passive methods tending to be less precise but having higher throughput.[15,75]

III. PLGA NP FUNCTIONALIZATION

A. NP loading

Nanoparticles can hold and deliver many different types of drugs and other substances including but not limited to doxorubicin (cancer),[19] paclitaxel (cancer),[9,12,39,76] Vascular endothelial growth factor (VEGF) (regenerative medicine),[77] and various nucleic acids (whatever they code for, numerous applications).[21,26,39,59,78] Drug/substance choice plays a major role in the determining fabrication method and based on whether the drug/substance is soluble in water or not. Doxorubicin and nucleic acids, for example, are water-soluble, whereas paclitaxel and VEGF are not.

The water-soluble drugs and substances must be dissolved in water, so for the emulsification-solvent evaporation method, double emulsion must be used.[19,21,26,39,59,78] Water-soluble drugs are, thus, in the first water phase before adding to the organic solvent phase and then the bulk water phase. If you dissolve the drug in the bulk water phase, the encapsulation efficiency will be very low as most drug will never see any PLGA, so there is a lowered chance the drug is encapsulated whereas if the drug is dissolved in the first water phase, then all the drug has the potential to see PLGA as it is all added to the organic solvent phase first. For the precipitation method, since the organic solvent and water dissolve readily, it is important to keep the volume lower to increase the encapsulation efficiency. The larger the working volume, the higher chance the drug never sees PLGA, and increasing the amount of organic solvent phase creates a new problem as more nanoparticles will then end up forming with no drug, which is harder to separate out at the end as they should all roughly be the same size. Therefore, a technique like the microfluidic HFF, as shown in Fig. 3, will lead to higher encapsulation efficiency since the working volume is much smaller than bulk mixing and the ratio of organic solvent to water can be carefully managed.

Drugs like paclitaxel and the cytokine VEGF on the other hand cannot be dissolved in water and, therefore, are dissolved in the organic solvent.[9,12,39,76,77] This means that the simpler single emulsion technique can be used for the emulsification-solvent evaporation method. Water-insoluble drugs are in the organic solvent phase, which is added to the bulk water phase, so all the drug has a chance to be encapsulated. Since the drugs are dissolved in the organic phase, simple bulk nanoprecipitation can be used for the precipitation method. If the microfluidic HFF technique is desired, the organic solvent phase should be switched with the water phase, so the organic solvent phase is in the middle channel and is squeezed by water phases on both sides. This would keep the drug, which is in the organic solvent phase, in the center of the channel and, thus, able to see PLGA. If you kept the organic solvent phase on the outside, then the formed nanoparticles on the water boundary would be more likely to contain no drug. The microfluidic method is still attractive compared to the bulk method due to its better experimental control, smaller working volume, and finer parameter tuning.

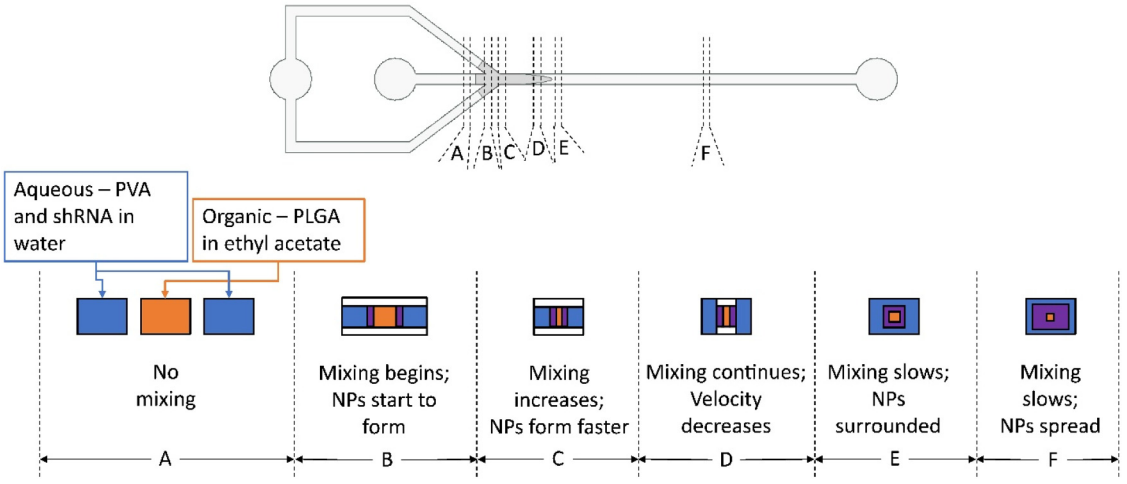

FIG. 3. Microfluidic 3DHFF nanoparticle fabrication steps. shRNA is dissolved in the outer aqueous phase and mixed with the organic phase where the channels meet. The water and acetonitrile go through solvent exchange on the barrier of the phases. After solvent exchange, some PLGA is now in a water-rich environment and so it self-assembles into a nanoparticle. Solvent exchange continues over the length of the channel wherever the aqueous phase meets the organic phase with the nanoparticles growing over the length of the channel. Adjusting the speeds of the channels and the ratio of speeds can tune the final size of the nanoparticles.

B. Surface modifications

PLGA nanoparticles themselves are simply a ball of PLGA and so they do not necessarily have control of where they are delivered, their surface charge, and the potential for collisions with other PLGA nanoparticles, resulting in aggregations or increased size. PLGA nanoparticles can have their surface modified with the addition of many different types of molecules including various targeting ligands, chitosan, lipids, polyethylene glycol (PEG), and surfactants as listed in Table II.

For PLGA nanoparticles to be effective, they must be delivered to the right cells in the body. Adding cell-targeting ligands via physical association or chemical conjugation allows the PLGA nanoparticle to be internalized by receptor-mediated endocytosis after ligand–receptor binding. Ligands can be added by either electrostatic interactions where a positively charged group attached to the ligand is attracted to the negatively charged PLGA surface or through hydrophobic interactions where a ligand attached to a hydrophobic chain is attracted to the hydrophobic PLGA surface.[79] Chemically, a ligand can be attached by carbodiimide (carboxyl–amine reaction), maleimide (maleimide–thiol reaction), or click chemistry (alkyne–azide reaction).[121,122] The most common ligands are antibodies that only interact with their specific surface antigen including anti-CD133 (gastric carcinoma cells),[9,79,80] anti-Prostate-specific membrane antigen (PSMA) (prostate cancer cells),[79,81] and anti-HER2 (ovarian and human breast cancer cells).[79,82–84] Other ligands include biotin (cancer),[79,85] bisphosphonates (bone-related conditions),[79,86–88] folic acid (brain, breast, cervical, colorectal, epithelial, kidney, lung, and ovarian tumors),[79,89] lectins (cancer),[79,90–93] mannan (macrophages and dendritic cells),[79,94] aptamers (designed against any cancer-related biomarker),[79,95–98] peptides (cancer and atherosclerotic plaques),[79,99–108] sialic acid (cancer, leukocytes, platelets, and endothelial cells),[79,109] and transferrin (breast cancer, pancreatic cancer, lung cancer, and brain gliomas).[79,110]

TABLE II. List of surface modifications. Additional secondary functions and examples in the text.

Surface modification	Main function(s)	Example(s)	Reference(s)
Ligands	Cell targeting	Antibodies Biotin Bisphosphonates Folic acid Lectins Mannan Aptamers Peptides Sialic acid Transferrin	9, 79–110
Chitosan	Adds positive charge to NP Improves cellular uptake	Chitosan	6, 14, 20, 31, 111
Lipids	Increase encapsulation efficiency Assist cellular uptake Encourage endosomal escape Can be charged	Phospholipid Cationic lipid Anionic lipid Cholesterol	59, 78, 112–118
PEG	NP shielding	PEG	8–10, 32, 35, 36, 78, 119
Surfactants	NP stabilization Can be charged	PVA Pluronics Polysorbates Poloxamers DMAB	5–9, 11–13, 17–19, 21–23, 26, 28–30, 34–36, 40, 120

Chitosan is a polycationic polymer made from chitin that has been used for numerous biomedical applications including being added on to existing nanoparticles and serving as the base polymer for nanoparticles. For PLGA nanoparticles, it is used to add a positive charge to the surface of the nanoparticles.[6,14,20,31,111] A positive surface charge on nanoparticles allows the nanoparticle to uptake into the cell more readily as charged nanoparticles (positive and negative) show preferential uptake in cells with positive charged nanoparticles showing the highest.[111,123,124] Chitosan can be added to the surface of PLGA nanoparticles through simple bulk mixing as the PLGA gives the nanoparticle a negative charge, which will attract the positively charged chitosan. Since chitosan is positively charged, any negatively charged drug that is in the bulk when it is added will be attracted to it. This could result in a negatively charged drug being entrapped onto the surface of the nanoparticle, as opposed to within the ball of PLGA. The release profile could then be negatively impacted and result in incorrect encapsulation efficiency reporting as the drug on the surface would be counted as the entrapped drug. This issue can be avoided by adding chitosan after nanoparticle formation so that no drug is in the bulk phase when the chitosan is added.

Many types of lipids have been added to PLGA nanoparticles to enable monodisperse fabrication, improve stability, increase encapsulation efficiency of nucleic acids, assist cellular uptake, and encourage endosomal escape of nucleic acids.[59,78,112–118] Phospholipids are types of lipids that are comprised of a hydrophilic "head" group containing a phosphate group, two hydrophobic "tails" that are derived from fatty acids, and a glycerol molecule to link them together. This amphiphilic nature causes phospholipids to self-arrange into a layer when in a solution containing water and organic solvent, as is the case when fabricating PLGA nanoparticles. The hydrophilic head will orient toward the water with the hydrophobic tails orienting toward the organic solvent. When surrounding a droplet of organic solvent, these phospholipids will self-arrange into a monolayer around the organic solvent forming a micelle. This will surround anything in that organic solvent droplet, which is the ball of PLGA for PLGA nanoparticles. The incorporation of

phospholipids, therefore, increases stability and circulation time. The addition of cationic lipids has been shown to increase the encapsulation efficiency of nucleic acids due to the positive charge on the lipid attracting the negatively charged nucleic acids.[59,78,113,117,118] Adding cholesterol maintains membrane integrity by matching the molar ratio to endogenous membranes as well as decreases the number of surface-bound proteins and improves circulation time.

PEG is a polyether compound that is commonly added to PLGA nanoparticle surfaces to help the nanoparticle avoid the mononuclear phagocytic system (functions as a shielding group). In short, this allows the PLGA nanoparticle to avoid phagocytosis and, thus, reach the intended target location. PEG is a common choice as a shielding group as it is electrically neutral, has high hydrophilicity, and shows high spatial repulsion.[8–10,32,35,36,78,119] PEG can be added via physical absorption (as part of a hydrophobic or charged group), covalent coupling (as part of a reactive group), or self-assembly (as part of a hydrophobic copolymer including lipids).[119] PEG has also been shown to improve the nanoparticle stability through steric repulsion, decrease the encapsulation efficiency of hydrophilic drugs, increase the encapsulation efficiency of hydrophobic drugs, and be susceptible to the accelerated blood clearance, which is a phenomenon where the circulating half-life of PEGylated nanoparticles is shortened after a second injection of nanoparticles is introduced, theorized to be the result of the spleen producing and releasing anti-PEG antibodies after the first injection.[59,78,112–117,119,125]

Surfactants are compounds that decrease surface tension between two materials, acting as a stabilizing compound. Examples include various pluronics,[8,30,40,120] polysorbates,[36,120] and poloxamers,[29,35,36,120] as well as the commonly used polyvinyl alcohol (PVA).[5–7,9,12,13,17–19,21–23,26,28,34,40,120] For PLGA nanoparticles, a surfactant is added to seal the nanoparticles, so they do not grow or aggregate. They consist of a hydrophilic head and a hydrophobic tail so when they interact with a nanoparticle, the tails orient toward the PLGA, and the heads orient toward the water. This results in the creation of a monolayer around the ball of PLGA. They can be further classified as nonionic and ionic depending on the charge of the head. Different surfactants also maintain different sizes of nanoparticles by changing the contact angle. Surfactants can also play a role in delivery as they can allow the surrounded PLGA core to avoid phagocytosis when attempting to reach the target area. The surfactant didodecyldimethylammonium bromide (DMAB) is unique as it has an inherent positive charge, thus removing the need for chitosan or other additions that add positive charge to the nanoparticle.[11]

IV. PLGA NP BIOLOGICAL CONSIDERATIONS

When PLGA nanoparticles enter the body, there are several major factors to consider. These being passive targeting, efficiency of cellular uptake, the release profile of any carried drugs, and the degradation/clearance/toxicity of the nanoparticle as seen in Table III. Accounting for all these factors will allow any nanoparticle to perform its function while not harming the body in any way.

Targeting can be controlled by adding surface modifications as mentioned above (active targeting), but targeting can also be done by controlling the size of the PLGA nanoparticle (passive targeting). There are cases where active targeting is undesirable such as to avoid creating a binding site barrier effect and to reduce toxicity.[126–128] In these cases, size control becomes very important in determining where the nanoparticles will uptake. Generally, nanoparticles must be within the 10–200 nm range as nanoparticles less than 10 nm in diameter are rapidly eliminated by the kidneys and nanoparticles greater than 200 nm in diameter cause inflammation and phagocytic activity.[129–132] Examples for specific cells are that sizes of 30–50 nm are desired when targeting pancreatic cancer cells, sizes of 40–60 nm are better for targeting breast cancer cells, sizes around 100 nm are optimal for targeting the gastrointestinal tract, and sizes around 120 for respiratory epithelium.[129,133–135]

TABLE III. List of biological considerations. Additional secondary factors and examples in text.

Biological considerations	Main factor(s)	Affect(s)
Passive targeting	Size	Where in the body NP uptake occurs
Cellular uptake	Size	Whether or not cellular uptake occurs
	Shape	Rate pathway of cellular uptake
	Surface modifications	
Release profile	Attached vs entrapped drug size	Drug release pathway and timing
Degradation and clearance	Shape	Circulation time, toxicity, and environmental effects
	Surface modifications	

Cellular uptake of PLGA nanoparticles can occur in a few ways depending on the size, shape, and surface modifications (including charge). Five common pathways for cellular uptake are phagocytosis, caveolin-mediated endocytosis, clathrin-mediated endocytosis, independent endocytosis, and macropinocytosis each being more likely for certain nanoparticle conformations.[129,136] Phagocytosis of nanoparticles occurs for larger particles like those greater than 200 nm and is initiated by opsonization (adsorption of antibodies onto the nanoparticle surface), which is then recognized by phagocytes and internalized.[132,137] Surface modification by adding PEG can act as a shield, essentially preventing opsonization and, therefore, phagocytosis.[137] Caveolin-mediated endocytosis happens where caveolin is located on the cell membrane. Uptake forms a 50–80 nm size vesicle, which can escape lysosomal degradation.[138] Clathrin-mediated endocytosis takes place where clathrin is located on the plasma membrane and creates vesicles with diameters of 100–150 nm but are more susceptible to lysosomal degradation.[139,140]

Independent endocytosis occurs in cells without clathrin and caveolae, and an example is folate-modified nanoparticles, which

also might escape trafficking into lysosomes.[141,142] Macropinocytosis is for large nanoparticles that are too big for caveolae- or clathrin-mediated endocytosis and consists of bulk fluid uptake of all particles and dissolved molecules in the extracellular fluid.[143] Nanoparticle shape affects uptake at certain sizes. For nanoparticles greater than 100 nm in diameter, the order of highest uptake to lowest was rods, spheres, cylinders, and then cubes.[144] For nanoparticles less than 100 nm in diameter, spheres showed the highest uptake.[145,146] Using needle-shaped PLGA nanoparticles was attempted and showed increased uptake but with the increased risk of lysosome disruption leading to cell apoptosis.[147] Although the size dictates the pathway, MacCuaig *et al.* have shown that active targeting (surface modification) has a much larger impact on uptake than passive targeting (size/shape control).[134] As mentioned above, adding certain surface modifications improves cellular uptake like the addition of antibodies or peptides when targeting cancers or certain lipids when targeting specific areas of the body.[134,148] Also from above, positively charged nanoparticles (through surface addition of chitosan) show preferential uptake as the cell membrane possesses a slight negative charge and, thus, there are some electrostatic attractions influencing cellular uptake.[123,124,149,150]

PLGA nanoparticles for delivery must consider the release profile after uptake into a cell. The desired speed at which the delivered drug is released is disease-dependent and so the nanoparticle should be tailored to each case. Generally for PLGA nanoparticles, the burst release of the drug is followed by a prolonged release as there is drug adsorbed on the surface, which releases quickly, and drug entrapped inside the core, which releases slowly.[151–156] Smaller nanoparticles intuitively degrade faster and, thus, deliver their drug faster.[157,158]

After the PLGA nanoparticle delivers its drug, it must then degrade or otherwise clear from the body to limit its toxicity. PLGA can be rapidly cleared by the mononuclear phagocyte system (MPS) as PLGA nanoparticles can diffuse across the permeable vasculature in this system.[159] Complete removal of PLGA nanoparticles has been shown to occur within 10 min of administration.[160,161] Based on this, if you are targeting rapid release to a location that does not take too long to reach, then this time constraint is of no concern. If you are targeting a location that takes more time, then you will need to employ techniques, some described above, to avoid this rapid clearance. Another way to avoid the MPS while also improving circulation time is to incorporate a hydrophilic shell to your nanoparticle, although the trade-off is increased risk of aggregation and liposome destruction.[119] Non-spherical nanoparticles show reduced immune clearance and improved blood circulation time compared to spherical nanoparticles.[162,163] The use of certain lipids or PEG can activate the host immune response or be cleared quickly after first administration due to the production of anti-PEG antibodies in a phenomenon known as the accelerated blood clearance effect.[117,119,164,165] To avoid this, biodegradable lipids can be used.[166–170] Nanoparticles with charged lipids can avoid clearance by the MPS but are susceptible to aggregation in solutions of high ionic strength like blood, which can result in rapid removal from the blood stream.[171,172] Nanoparticles made with stabilizing agents such as chitosan, PVA, and Poloxamer 188 showed limited toxicity.[173] Additions of nonionic surfactants like Poloxamer 188 also embrace the stealth effect by which opsonization and, thus, phagocytosis is avoided.[120]

The use and disposal of PLGA nanoparticles inevitably releases some of them into the environment, leading to potentially negative effects if in a high enough concentration.[174,175] They can enter the environment by photochemical transformation, oxidation and reduction, dissolution and precipitation, adsorption and desorption, combustion, biotransformation, and abrasion.[176] Material flow analysis and environmental fate modeling detail when PLGA nanoparticle emissions are produced, where they are ultimately deposited, and what happens to them once they are released to the environment.[175,177,178] Negative effects of environmental release include nuclei damage and oxidative stress in microorganisms, DNA folding, chromosomal aberrations, and oxidative stress in plants, liver inflammation, reproductive toxicity, and oxidative stress in animals, and shape-based toxicity and oxidative stress in humans.[179–186]

V. CONCLUSION

PLGA nanoparticle applications grow constantly due to its flexibility in adapting the original bare PLGA nanoparticle by considering new drugs, surface modifications, and biology, as well as the fabrication methods to make those adaptations possible. Continued efforts to expand the field to treat numerous diseases by delivering many new drugs maintain the relevance of research in this field.

The use of nucleic acids as the loaded drug opens the applications of these nanoparticles to almost anything as the design of the nucleic acid can target almost anything. Some challenges remain like the ability to load drugs efficiently and evenly into PLGA nanoparticles with high throughput, the potential to scale up the high throughput processes like microfluidics that have been shown to raise the encapsulation efficiency ceiling, and the means to properly target the nanoparticles so they only go to the target region and nowhere else. As researchers look toward these and other new applications, PLGA nanoparticle fabrication, functionalization, and biological considerations are necessary starting points.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Eric K. Marecki: Conceptualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Kwang W. Oh:** Supervision (equal); Writing – review & editing (equal). **Paul R. Knight:** Supervision (equal); Writing – review & editing (equal). **Bruce A. Davidson:** Supervision (equal); Writing – review & editing (equal).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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