PLGA hybrid Nanoparticles: A promising system for drug delivery

Preeti Sangwan
M. Pharmacy (Pharmaceutics)

INTRODUCTION

PLGA is biodegradable polyester used for the preparation of NPs for various molecular targets. It is synthesized by random ring-opening co-polymerization of copolymerization of glycolic acid and lactic acid in the presence of tin (II) 2- ethylhexanoate, tin (II) alkoxides or aluminum isopropoxide (1). It is one of the most widely used biocompatible and biodegradable polymers and has been approved by FDA for developing therapeutic device, due to its minimal systemic toxicity. PLGA can be hydrolyzed in the body and yield biodegradable lactic acid and glycolic acid and these hydrolysis products get metabolized in TCA cycle. There is very minimal systemic toxicity associated by using PLGA for drug delivery or biomaterial applications as the body very effectively deals with these two monomers. To develop a successful nanoparticulate system, drug release and polymer biodegradation are two important consideration factors that are mainly governed by the physicochemical properties of the polymer.

Various factors like surface modification of PLGA, drug encapsulation methods and particle size, additives added during formulation, molecular weight of drug, ratio of lactide to glycolide moieties has strong influence on the release and effective response of formulation. PLGA monomers with acidic nature are not suitable for drugs or bioactive molecules (2). PLGA NPs, due to their ability to entrap both water soluble and water-insoluble molecules, are in process of extensive evaluation for the delivery of drugs, genetic materials and proteins to cultured cells and experimental animals.

These nanoparticulate systems when endocytosed by cells release their therapeutic payload by both passive diffusion and slow matrix degradation (3). They posses several attractive features such as small size, high structural integrity, stability and controlled release capability which makes them highly attractive as therapeutic delivery vehicles. In addition, they are also easy to fabricate with tunable properties and readily functionalized for targeted as well as smart drug delivery. These systems have been extensively investigated for sustained and targeted/localized delivery of various agents, including drugs, proteins and peptides, and recently plasmid DNA, owing to their ability to protect DNA from degradation in endolysosomes (4, 5, 6, 7). Lupron Depot® is a commercially available PLGA based drug delivery device for the treatment of advanced prostate cancer. Thus, we can say that PLGA NPs have great potential in many clinical applications currently under active investigations.

Hydrolysis of PLGA

PLGA polymers can offer long-term release of their contents in a recurring, pulsatile manner, the primary focus of past studies has been in using them to replace the multiple immune boosting administrations typically required to induce protective immunity. As a controlled delivery system, PLGA NPs has potential to deliver antigens or adjuvants to a desired location at predetermined rates and durations, effectively regulating the immune response over a period of time (8). Also, they have been exploited to improve the oral bioavailability of numerous drugs having poor enzymatic/metabolic stability. In a study M.M. Yallapu et al. (2010), showed that curcumin loaded PLGA nano-formulation demonstrated increases in the cellular uptake performed in cisplatin resistant A2780CP ovarian and metastatic MDA-MB-231 breast cancer cells, respectively, compared to free curcumin (9). In other study, Yousef et al. showed that naproxen–PLGA NPs were able to improve the physicochemical characteristics of the drug and increased the anti-inflammatory effects of drug following its ocular or intra-joint administration (10).

PLGA offer a promising alternative strategy for peptide-based cancer vaccines. In 2012, Silvia et al. developed PLGA NPs as delivery systems to improve the potency of synthetic long chain peptide cancer vaccine. This nanoparticulate delivery system was able to enhance CD8+ T cell activation in vitro (11). Studies investigated PLGA NPs as potential drug carriers for oral insulin delivery. Their physicochemical characteristics, in vitro drug
release and hypoglycemic effects in diabetic rats were evaluated (12, 13). To maintain the long-term complications associated with diabetes mellitus a specific formulation of 1.6% zinc insulin in PLGA with fumaric anhydride oligomer and iron oxide additives has been found effective for oral administration.

This formulation has shown to improve the efficacy of intraperitoneally delivered zinc insulin and is able to control plasma glucose levels when faced with a simultaneously administered glucose challenge (14). In 2009 Saltzman and coworkers developed a surprisingly simple alternative delivery strategy based on PLGA polymer for siRNA delivery for in vivo gene silencing therapy (15). The results showed that a single dose of high amount siRNA-loaded NPs to the mouse female reproductive tract caused efficient and sustained gene silencing. PLGA NPs loaded with cyclosporin showed sustained release in the plasma with concomitant decrease in the cyclosporine associated nephrotoxicity whereas commercial formulation Sandimmune Neoral failed to achieve both (16).

The development of drug resistance is a major obstacle of cancer treatment. To counteract this problem, PLGA NPs-mediated simultaneous and targeted delivery of paclitaxel and tariquidar (inhibitor of P-glycoprotein) to tumour drug resistance has been studied by Patil et al. (17). The in vitro antitumoural activity of PLGA NP formulation incorporating paclitaxel has also been assessed on a human adenocarcinoma cell line and human small-cell lung cancer cell line and compared with the in vitro antitumoural activity of the commercial formulation by Feng et al. and Fonseca et al., respectively (18, 19). The biodistribution studies of PLGA nanoparticulate delivery system showed higher accumulation of diagnostic or therapeutic agents by the enhanced permeability and retention effect. Studies have revealed that when indocyanine green was delivered through NPs in healthy mice using a fluorometric assay method, the NPs led to higher indocyanine green deposit in organs (two to eight times) as well as in blood (five to ten times) compared with free solution, indicating the enormous potential of PLGA NPs as a delivery system for indocyanine green for its use in tumor diagnosis and photodynamic therapy (15).

This effect is enhanced by modifying the surface of the NPs with poly(ethylene glycol/oxide) (PEG). Coating the nanoparticle surface with a hydrophilic polymer, such as PEG, has been shown to confer long circulation properties to PLGA NPs. The presence of the hydrophilic polymeric chains on the surface of the NPs sterically stabilizes them against opsonization and subsequent phagocytosis (20). According to needs of its application and type of drugs to be encapsulated various methods have been used to synthesize the NPs. The solvent evaporation, interfacial deposition, the emulsification–diffusion and the nanoprecipitation are the various methods that can be used to prepare PLGA NPs (2). Generally in emulsification–diffusion method, the PLGA polymer is dissolved in organic solvent (ethanol, butyl alcohol, etc.). This organic phase is poured and separated in aqueous phase having stabilizer and subsequently emulsified by homogenizer. In solvent evaporation method, the polymer dissolved in volatile organic solvent such as dichloromethane, acetone, chloroform, ethanol, etc. is poured into continuously stirring aqueous phase with or without emulsifier/stabilizer and sonicated for a specified period of time.

Interfacial deposition methods have been used to synthesize both nanocapsule and nanospheres. In this method the NPs are synthesized in the interfacial layer of water and organic solvent (water miscible) and finally these NPs are separated by centrifugations. Nanoprecipitation is the most commonly used method for the preparation of PLGA NPs. In this method polymer dissolved in acetone is added dropwise into continuously stirring aqueous phase with or without emulsifier/stabilizer and consequently organic phase is evaporated under reduced pressure.

**Limitations of PLGA NPs (4, 10):**

Besides several advantages, there are certain limitations associated with PLGA-based NPs in terms of physicochemical and biological properties which restrict its applications in nanomedicine.

- PLGA-based NPs often present high encapsulation efficiency (EE); but the drug loading is usually poor.
- Leakage during storage.
- High burst release of drug from NPs leads to a loss of efficacy.
- Less circulation time in the body.
- Rapid uptake by reticuloendothelial system.
- The disadvantage associated with PLGA is the production of acids upon degradation, as is the case of many other biodegradable polymers. Several methods have been investigated for the stabilization of acid-sensitive drugs.
- The production of GMP PLGA with well defined properties can be expensive.
- Other limitation for the commercialization of NPs is the scaling-up.
Hybridization of PLGA NPs

Several limitations associated with using simple PLGA NPs can be alleviated by hybrid PLGA NPs. The art of hybridization has emerged as promising platform for various therapeutic applications. These novel hybrid drug delivery systems have an enormous impact on medical technology, and are greatly improving the performance of many existing drugs. Hybrid PLGA NPs posses numerous advantages over conventional NPs such as less cytotoxicity, biocompatibility, improved stability, improved drug release behavior, etc. These hybrid NPs are mainly prepared by singleor double-emulsion solvent evaporation (single emulsion for hydrophilic drug and double emulsion for lipophilic drugs) as well as nanoprecipitation method. Drug can be added either during the time of nanoparticle preparation or can be adsorbed on the surface of the NPs after their production (4, 6).

PLGA-lipid nanohybrids

Lipid PLGA nanohybrids are the unique nanosystems that combine the mechanical advantages of biodegradable polymeric NPs and biomimetic advantages of liposomes, have emerged as a robust and promising delivery platform for the delivery of various drugs. Core–shell type lipid–polymer hybrid NPs, can provide advantages such as controllable particle size, surface functionality, high drug loading, entrapment of multiple therapeutic agents, tunable drug release profile, and good serum stability (21). This type of NPs mainly consists of three distinct functional components: (i) a hydrophobic polymeric core in which poorly water-soluble drugs are incorporated with high loading yields; (ii) a lipid layer surrounding the core which acts as a highly biocompatible shell and as a molecular fence and hence promote drug retention inside the polymeric core; and (iii) a hydrophilic polymer stealth layer outside the lipid shell to promote nanoparticle stability and systemic circulation lifetime.

The polymeric core and the lipid shell are held together due to hydrophobic interactions, vander waals forces, electrostatic interactions or other non-covalent forces whereas hydrophilic polymer stealth layer is often conjugated to the lipid shell through covalent bonds (22). These nanohybrids can be conjugated with appropriate targeting ligands such as aptamers, folic acid, transferrin, antircarcinombryonic antigen half-antibody, or single chain tumor necrosis factor 59 to deliver NPs at the target tissues for treating cancers (22). Particles smaller than 100 nm (similar to virus-like architecture) are promising for intracellular drug targeting and vaccine adjuvants (22). These hybrid NPs mainly consists of PLGA core coated with single or multiple layer of lipids that may be zwitterionic, cationic, anionic, and neutral phospholipids such as lecithin, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). Wean et al. showed that Levofloxacin loaded hybrid NPs exhibit higher antibacterial efficacy against biofilm cells.

These NPs were more stable than simple PLGA NPs and presence of lipid coat in the hybrid NPs resulted in slower antibiotic release rates (23). PeiQi et al. showed that paclitaxel loaded folic acid targeted lipidshell and polymer-core NPs on intravenous administration led to tumor regression and improvement of animal survival in a murine model, compared with that observed With Taxol and biodistribution study showed that paclitaxel concentration in tumor for paclitaxel encapsulated NPs was higher than other paclitaxel formulations (24). Chan et al. developed a “nanoburr” system for the treatment of injured vasculature, by conjugating the lipid-coated PLGA hybrid NPs with a novel peptide ligand, screened from a combinatorial library of heptapeptide ligands against human collagen IV, which represents 50% of the vascular basement membrane (25). This “nanoburr” system demonstrated efficient targeting toward vascular basement membrane, high NPs accumulation in the region of injured vasculature in a rat model, and sustained drug release over 2 weeks.

PLGA-polymer nanohybrid

To resolve the constraints concerned with PLGA NPs, PLGA with other polymers have shown promising results. In context of PLGA nanocomposites using other polymer, Sanna et al. (2013) designed resveratrol loaded NPs using poly(epsilon-caprolactone) and PLGA–PEG blend for prostate cancer treatment (13). The resulted NPs were efficiently taken up by PCA cell lines and significantly improved the cytotoxicity compared to that of free resveratrol. Noha et al. showed that chitosan-coated PLGA NPs offer a flexible technology platform for DNA/RNA delivery. These particles effectively bind the antisense RNA and are taken up into the cells, which are essential requests for DNA/RNA delivery.
PLGA-inorganic nanohybrid

More recently, PLGA-inorganic nanohybrid delivery system has also gained popularity in delivery of several bioactive agents. Paclitaxel loaded polymer-metal hybrid NPs have been developed which serve as new class of core-shell theranostics. The NPs were fabricated by loading paclitaxel in PLGA followed by deposition of silver in presence of polyvinylpyrrolidone and then growing silver-gold shells around the drug loaded core so as to form polymer core and metallic shell hybrid NPs (26). In other study, Gajendiran et al., reported the novel synthesis of citrate PEG hybrid dendron stabilized gold NPs with linearly linked PLGA-PEG-SA-PEG-PLGA multiblock copolymer for the delivery of rifampicin, an antitubercular drug (27). The drug loading and drug content in gold nanoparticle conjugated multiblock copolymer NPs was found to be greater than reported literature.

PLGA-oil nanohybrids (PONHs)

It is a novel approach in which highly lipophilic drug pre-solubilized in oil phase is dispersed in polymeric matrix. This hybrid system integrates the advantages of lipid-based (e.g. efficient encapsulation of highly lipophilic drugs) and polymeric colloidal carriers (e.g. uniform size, good stability). This nanosystem improves the potential therapeutic benefits of poorly water-soluble drug. For the first time in 2013 Naverkar et al., developed all-trans-retinoic acid and indomethacin loaded polymer-oil nanohybrid. This nanocarrier system substantially increased the EE and significantly improved the anticancer activity of the drug over the standard PLGA only NPs (28).

Other PLGA nanohybrids

Combination of different materials with PLGA has recently become one of the most innovative tool in discovery of new delivery system. Several polycations that have been used for cationic surface modification includes polyethyleneimine, cetyltrimethylammonium bromide, poly(2-dimethyl-amino) ethyl methacrylate, didodecyl dimethyl ammonium bromide, and chitosan (5). These polycation have potential to improve the delivery application of PLGA NPs and emerged as a novel approach to functionalize the surface of the NPs. In other approach Aravind et al. developed aptamer conjugated paclitaxel and magnetic fluid loaded fluorescently tagged PLGA NPs for targeted cancer therapy. Aptamers are single-stranded DNA or RNA oligonucleotides with well-defined, three-dimensional structures. Aptamers can recognize a wide variety of molecules (e.g, proteins, phospholipids, sugars, and nucleic acids) with high affinity and specificity. This aptamer mediated delivery resulted in enhanced binding and uptake to the target cancer cells and exhibited increased therapeutic effect of the drug (29). Various other method like conjugation of PLGA with cholic acid, transferin and GI dendrimer have been explored to improve in vivo stability, increase drug loading and to control drug release behavior (6, 7).

PLGA hybrid NPs are one of the most innovative and noninvasive approaches for the drug delivery to the central nervous system. Surface modified PLGA NPs can easily cross the blood–brain barrier and deliver the drugs to exert their pharmacological activity in the central nervous system (6). Novel PLGA nanohybrids like PLGA-oil nanohybrid, clay PLGA nanohybrid and PLGA-protein hybrid has shown promising results in potentiating the insufficient loading of simple PLGA NPs. Mayuri et al. formulated polymer-oil nanostructured carrier for the first time for controlled delivery of highly lipophilic drug all-trans-retinoic acid and indomethacin. This hybrid nanocarrier system substantially increased the EE of alltrans-retinoic acid and indomethacin by up to 259% and 124%, respectively. Incorporation of oil facilitated more uniform distribution of the drug molecules which subsequently led to improved drug release kinetics with significantly reduced burst release effects (28).

Applications of hybrid PLGA NPs

These hybrid PLGA NPs have been investigated for sustained and targeted/localized delivery of different agents, including drugs, proteins and peptides and, recently, plasmid DNA, owing to their ability to protect DNA from degradation in endolysosomes (5, 8).

- These NPs can be used for the delivery of growth factors to the tissue-engineered vascular graft and enhance the interaction between vascular cells and the graft scaffold, thereby modulating vascular graft healing and remodeling. This drug delivery system extended the period of drug release, thus reducing initial burst release and protected the encapsulated agent from enzymatic degradation (8).
• These nanoparticulate delivery systems could be used for localizing therapeutic agents or gene delivery into endothelial cells or other types of cells for improving the design of tissue engineered grafts. PLGA NP-coated stents can effectively deliver genes or drugs to vessel walls and also improves DNA controlled release (5, 15).

• Due to large surface areas and functional groups PLGA hybrid NPs can be conjugated to multiple diagnostic (e.g., optical, radio isotopic or magnetic) agents. These particles were implanted in cancer patients for early cancer detection and screening. This technology has been widely used in diagnosis and treatment of cancer (30).

• PLGA micro bubbles have been developed as ultrasound contrast agents for improving ultrasound imaging in the diagnosis of cardiovascular disease and tumors. These systems may also offer enhanced sensitivity and specificity for in vivo tumor imaging (8, 30).

• Encapsulation of several anticancer drugs such as paclitaxel, cisplatin and hypericin, in the hybrid PLGA NPs strongly enhances their antitumoral efficacy in comparison to the free drugs (14, 24, 31).

• NPs of different sizes within their range can (given in theory) are injected systematically to target specific immune-response modulators, such as dendritic cells and macrophages, both in vitro and in vivo, rather than only being useful in a localized immunization (20).

• These nanosystems effectively enhance immune responses and have been observed to stimulate the immune response as measured by an increase in IL-2 and IFN-γ in spleen homogenates (8).

REFERENCES


