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REVIEW PAPER

Biobased Polylactide Nanoparticles as Novel Gene Delivery Nanoplatforms: Can They Be Used for DNA Transport?

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ABSTRACT

Gene therapy is a rapidly progressing field with vast prospective for fundamentally treating human diseases such as cancer, damaged tissues, and *genetic syndromes*. Between various approaches of gene delivery, there is a growing interest in oral administration of DNA as one of the safest and most straightforward methods. Nanoparticles are some of the important examples of nano-materials for molecule delivery (drugs, growth factors and DNA) used in biomedical applications. Several researchers have revealed the process of nanotechnology, specifically polymeric nanoparticles, as DNA delivery structures for transdermal routines. Polylactic acid (PLA) and its famous co-polymer polylactic-co-glycolic (PLGA) are biocompatible synthetic polymers widely used to produce nanoparticles. Biobased, biosourced, biodegradable biocompatible, and bioabsorbable polylactide nanoparticles are one of the most promising materials in gene therapy serving as DNA delivery vehicles. Polylactide nanoparticles are easily processable and undergo degradation into natural metabolites while matching its degradation rate with the healing time of damaged human tissues. This mini review presents the new developments in the applications of polylactide nanoparticles as DNA delivery systems. In addition, the release of DNA from these nanoplatforms will be reported briefly.

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INTRODUCTION

Gene

A *gene* is the basic physical unit of heredity, a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA; upon translated into protein, this process leads to the expression of hereditary character [1, 2]. DNA is the molecule that carries genetic information for the development and functioning of an organism. Deoxyribonucleic acid is a polymer composed of two polynucleotide chains that coil around each other to form a double helix (**Scheme 1**)[3].

Gene Therapy

Gene therapy is a rapidly advancing field with vast prospective for treating human diseases such as *cancer, damaged tissues* and *genetic syndromes* basically [4]. For achieving effective gene therapy in a scientific context, it is imperative that gene-delivery structures are nontoxic, easy to use, and effective in delivering healing transgene-expression [5]. Over the previous years, several explorations with viral-vectors have recognized the gold-standard for positive gene-transfer and elevated expression in objective cells [6, 7]. However, the future tendency leans toward the advancement of developed approaches for non-viral gene-transfer, owing to the substantial immunogenicity associated with the usage of diseases [8]. Non-viral vectors are principally appropriate, as they permit simplicity of important manufacture and are comparatively less *immunogenic* (causing or capable of producing an immune response) [1, 9]. Newly, numerous unique non-viral vectors have been advanced that

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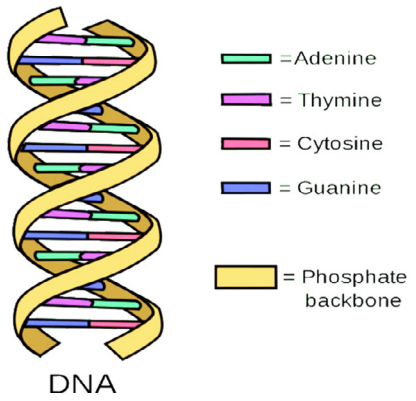


Scheme 1. Structure of DNA.

Table 1. Application of nanoparticles in gene therapy.

Type of Nanoparticles	Applications	
Gold/Poly-amido-amine Nanoparticles	Gene Therapy (Novel non-viral vector)	[16]
SiO_2@LDH Nano-particles	Improved Immune/Response of Hepatitis B/Virus	[17]
Poly-Ethylenimine/Plasmid DNA Nanoparticles	Gene Therapy	[17]
Hyaluronic Acid/Chitosan/Plasmid DNA Nanoparticles	Encoding TGF-ß1 , Controlled Releasing of DNA	[18]
Meso-porous Silica Nanoparticles	Accelerating Achilles tendon Therapeutic via PDGF gene delivery	[19]
Chitosan/Plasmid DNA Nanoparticles	Tissue Repairing	[20]
Fe_3O_4@SiO_2 Nanoparticles	Gene Therapy, Cellular Imaging	[21]

approach diseases with respect to transfection-efficiency [10].

Polymeric nanoparticles for transdermal delivery of DNA in gene therapy

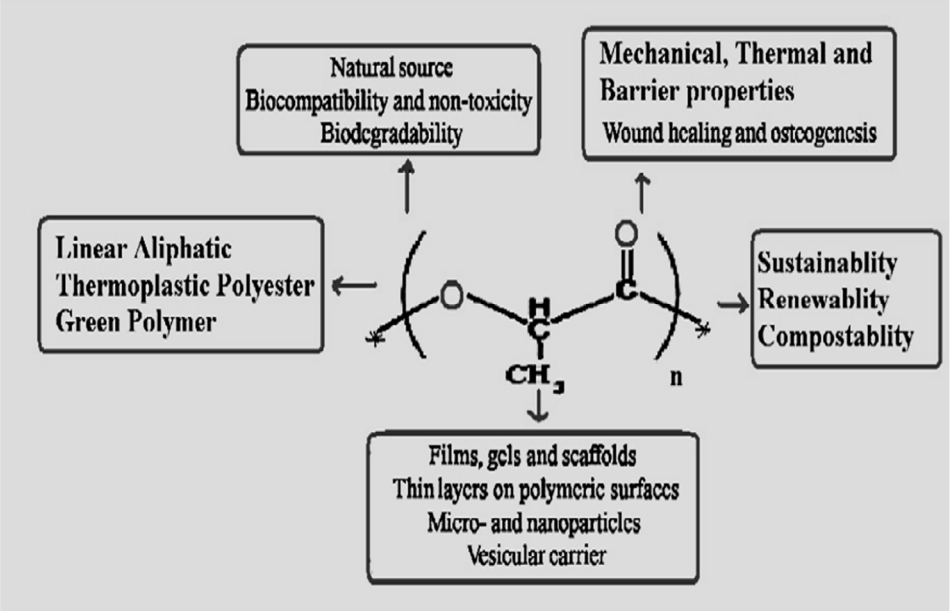
The capability to combine genes into a functionalized nanoparticle proves a novel era in pharmacotherapy for selectively delivering genes. A range of non-viral delivery systems that can be applied in various scientific situations are also obtainable, and one hopeful route is the progress of biodegradable, echogenic nanoparticles [1, 11, 12]. Polymeric nanoparticles have been proved to be the most promising vehicles for clinical gene therapy due to their tunable size, shape, surface, and biological behaviours. Due to their small sizes, nanoparticles can simply interact with biomolecules on the cell surface or inside cells and deliver genetic materials such as DNA. Biodegradable polymeric nanoparticles can be conjugated with *genetic* material (like DNA) via electrostatic attraction at physiological pH, thereby facilitating gene delivery [13-15]. **Table 1** displays some types of nanoparticles used in gene therapy.

A biodegradable polymer is a polymer which is submitted to the degrading procedures *in vivo* [22-26]. The polymeric nanoparticles commonly provide an extended surface area, high drug loading capability, feasibility of functionalization with ligands, controlled drug releasing capacity, minimal toxicity, biocompatibility, storage stability, and flexibility in the management methods [12, 27, 28]. Furthermore, these nanostructure materials signify unique ground breaking non-invasive methods for delivery structures in biomedical fields such as gene-delivery materials.

PLA (poly(lactic acid) or polylactide) with the chemical formulation of $(C_3H_4O_2)_n$ is one of the significant biodegradable polymers which can be derived from 100% renewable bio-resources like rice and wheat through fermentation and polymerization (**Scheme 2**) [29-31]. The strategic advantages of polylactide include lower energy consumption required and lower greenhouse gas emission throughout production [26, 32, 33]. Polylactide biodegrades to water and CO_2 at the end of its life cycle [12, 34].

PLLA, PDLA and PDLLA

PLA holds stereo-isomers like Poly (L-Lactide) (PLLA), Poly(D-Lactide) (PDLA) and Poly(DL-



Scheme 2. Polylactide: A biodegradable polymer [26].

Lactide) (PDLLA) which have drawn vast attention in biomedical uses due to its special biocompatibility and mechanical specifications [12, 30, 35, 36].

Biodegradation of polylactide in the human body

v Polylactide can undergo scission in the human body [26].

v Polylactide degrades to monomeric units of lactic acid as a natural intermediate in carbohydrate metabolism [37, 38].

v Polylactide degradation rate matches with the healing time of damaged tissues [39].

v However, its long degradation times together with the high crystalline nature of its fragments could result in inflammatory reactions in the body [26, 40].

Biobased biodegradable biocompatible bioabsorbable polylactide nanoparticles are one of the most promising materials in gene therapy as delivery structures for DNA [41, 42]. This is because these biodegradable nano-materials can be conjugated with DNA via electrostatic attraction at biological pH, hence facilitating gene delivery. Moreover, they can simply interact with biomolecules on the cell surface or inside cells (owing to the small size) and deliver DNA.

Thus, this mini review introduces the recent advances in the applications of polylactide nanoparticles as gene delivery systems. Additionally, the current challenges and future directions in this field will be reported briefly.

APPLICATION OF POLYLACTIDE NANOPARTICLES IN GENE THERAPY

For DNA-delivery usages, PLA, PLLA, PLDA, PDLLA and PLGA nano-particles have been exploited in an enormous variety of systems, like nano-particles incorporated in plants, nano-particles covered with other polymers, nano-particles containing chemical additives, porous nano-particles combined with growth factors which will be described fully below.

In a novel work in 2020, nanoparticles were manufactured by means of PLGA for encapsulating and delivering a model CRISPR–Cas9 plasmid to bone marrow derived macrophages. DNA release amounts were conducted at 3 various pH values (pH 7, 6, 4.5) to simulate different pH environments that the particles would experience throughout incubation in the media (pH 7.4) outside the cell, through initial (pH 6.8–6.1) and late endocytosis (pH 6.0– 4.8), and in lysosomes (pH 4.5) inside the cell. The quantitative analysis indicated that the maximum amount of DNA was released in the initial 24 hours [43].

PLA/PEG/PLA and PLA/PEG/PLA/PEI nanoparticles with various amounts of PEI were designed for delivering the DNA into MCF-7 cells by Hamidi *et al* [44]. The flow cytometer analysis revealed that by increasing the mass ratio of PEI:(PLA/PEG/PLA) (w/w%) in PLA/PEG/PLA/PEI/DNA nanoparticles, the efficiency of the gene delivery into MCF-7 cells was increased.

Table 2. Cumulative release of DNA from polylactide nanoparticles.

PLA nanoparticles	Released DNA (%) (According to the number of days)								Ref.
	1 day	3 days	5 days	7 days	10 days	15 days	20 days	28 days	
PLA/PEG	49.36±2.83	….	78	….	95	98	100	….	[46]
PLA/PEG/PLA	15	….	….	….	….	….	….	….	[46]
PLA/PEG/Chitosan/FA	45.5	….	….	….	….	….	….	….	[51]
PLA/PEG/PLA without PEI	16	20	21	22	23	25	27	30	[52]
PEI:(PLA/PEG/PLA) ratio of 10:300 (% w/w)	73	72	73	75	78	82	80	85	[52]
PEI:(PLA/PEG/PLA) ratio of 5:300 (% w/w)	55	63	65	67	70	73	80	83	[52]
PEI:(PLA/PEG/PLA) ratio of 15:300 (% w/w)	75	81	82	83	85	86	90	92	[52]
PEI:(PLA/EG/PLA) ratio of 1:300 (% w/w)	35	43	47	50	51	53	54	60	[52]
PLGA	0.75	….	….	….	….	….	….	….	[43]

In another investigation Poly(D,L lactic-co-glycolic acid) nanoparticles were applied as trans mucosal DNA nano-carriers [45]. Particular benefits of these nanoparticles for in *vivo* gene delivery include: (1) their acceptable DNA loading capability, (2) their capacity to control the release of the encapsulated DNA for prolonged periods of time while preserving its slight conformational structure as well as its organic action, and (3) their ability to overcome the nasal mucosa barrier and transport the associated model DNA vaccine.

In another novel work in 2020, PLA/PEG/PLA nanoparticles were manufactured with the double emulsion solvent evaporation technique for DNA delivery into mammalian cells. The electrophoretic analysis demonstrated that the PLA/PEG/PLA could protect DNA from ultrasound damage and nuclease degradation [12].

The purpose of that novel work was to formulate and evaluate cationic polylactide/poly(ethylene glycol) (PLA/PEG) nanoparticles as novel non-viral gene delivery nano-device. Cationic PLA/PEG nanoparticles were prepared by the nano-precipitation method. The gene loaded nanoparticles were obtained by incubating the report gene pEGFP with cationic PLA/PEG nanoparticles. The physicochemical properties (e.g., morphology, article size, surface charge, and DNA binding efficiency) and biological properties (e.g., integrity of the released DNA, protection from nuclease degradation, plasma stability, in vitro cytotoxicity, and in vitro transfection ability in Hela cells) of the gene loaded PLA/PEG nanoparticles were evaluated, respectively. The obtained cationic PLA/PEG nanoparticles with high binding efficiency (95%) could protect the loaded DNA from the degradation by nuclease and plasma. The nanoparticles displayed sustained release properties in vitro and the released DNA maintained its structural and functional integrity. It also showed lower cytotoxicity than Lipofectamine 2000 and could successfully transfect gene into Hela cells even in the presence of serum. It could be concluded that the established gene loaded cationic PLA/PEG nanoparticles with excellent properties were promising non-viral nano-device with the potential to make cancer gene therapy achievable [46].

Kim *et al.,* [47] used PLGA nano-particles as gene-delivery systems for cartilage repair and regeneration. PLGA nano-particles mediated pDNA-SOX9 delivery in human mesenchymal stem cells (hMSCs) and prompted chondrogenesis in female mice by means of subcutaneous implantation model. Li *et al.* [48], examined the manufacture and in-vitro effectiveness of PDLLA Gene Transfection-Systems. PDLLA was employed to prepare nano-particles with a size range of 40–80 nm. PDLLA nanoparticles were applied for loading DNA via an adsorption process, but they failed to offer respectable expression records [48].

CUMULATIVE RELEASE OF DNA FROM POLYLACTIDE NANOPARTICLES

The physical stability of gene delivery systems is related to their in vitro efficiency, as it determines how the DNA-load is delivered to the target sites. Therefore, DNA release assay is investigated after different periods [49, 50]. **Table 2** displays the cumulative release of DNA from different polylactide nanoparticles according to the following equation:

% Released = [mass DNA released into solution]/[initial total mass DNA encapsulated]

The *in vitro* release profile of DNA from PLA nanoparticles revealed an initial burst release

within the first 24 hours, followed by a gradual increase. Similar research has indicated that the reason for the burst release of DNA is the release of DNA in the external surface of particles rather than encapsulated DNA in the central of particles [53-56].

LIST OF SYMBOLS

PEI : Polyethylenimine

FA : Folic acid

PLA/PEG/PLA : Poly (lactide)/Poly(ethylene glycol)/Poly(lactide)

DNA : Deoxyribonucleic acid

CONCLUSION

A novel biodegradable polymer (poly(lactic acid) or polylactide) is a polymer which undergoes the degrading procedures *in vivo*. Polylactide nanoparticles are one of the most promising materials in gene therapy as proper delivery structures for DNA. The up-to-date expansions in the usage of PLA nanoparticles as gene delivery systems have been studied here. Different kinds of PLA nanoparticles have been advanced to be employed in gene therapy fields.

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CONFLICT OF INTEREST

The author reports no potential conflict of interest.

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