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Aptamer-Conjugated PLGA Nanoparticles for Delivery and Imaging of Cancer Therapeutic Drugs

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Abstract

Most problems associated with chemotherapeutic agents involve non-specific cytotoxicity, low intratumoral accumulation and drug resistance. Targeted drug delivery systems (TDDS) based on nanoparticles (NPs) are a new strategy for better therapeutic efficiency, along with reduction of side effects commonly seen with cancer drugs. Poly (lactic-co-glycolic acid) (PLGA), as one of the furthest developed synthetic polymer, has gained significant attention because of excellent properties—including biodegradability and biocompatibility, controlled release of drug, protection of drug or gene from decomposition and ability to modify surface with targeting agents for both cancer diagnosis and therapy. Aptamers are single-stranded RNA or DNA that can fold through intramolecular interactions into specific three-dimensional structures to selectively and exclusively bind with interested biomarkers. In this review, we explain the latest developments regarding the application of aptamer-decorated PLGA NPs in delivery of therapeutic agents or cancer-related genes into cancer cells. Additionally, we discuss the most recent efforts in the field of aptamer-grafted PLGA-based NPs as theranostics and stimuli-responsive agents.

Keywords

Aptamer, Cancer therapy, PLGA nanoparticles, Targeted drug delivery

1. Introduction

Common issues with chemotherapeutic agents include non-specific cytotoxicity to normal cells, low intratumoral accumulation and drug resistance [1]. Nanotechnology has provided a new approach for improved therapeutic efficiency with reduced side effects and cancer drugs that are not toxic to normal tissues [[2], [3], [4]]. Nanoparticles (NPs) could transfer the therapeutic molecules in a controlled and sustained behavior, maintaining the therapeutic index over time, and avoid or decrease the need for repeated administration [5].

Nanoparticles can target the cancer cells either through active or passive targeting. Active targeting assists NPs by precisely selecting the target cancer cells in comparison to having them accumulate passively at the targeted site [6,7]. During recent years, many advances have been developed in the field of targeted drug delivery systems (TDDS) based on nanoparticles to identify the specific cancer molecular markers, which resulted in the improvement of selective cellular binding, as well as internalization to cells using receptor-mediated endocytosis [8]. These targeting delivery systems include hydrogel and polymeric NPs [[9], [10], [11]], calcium carbonate NPs [12] lipid-based NPs [13], mesoporous silica NPs (MSNs) [14], gold NPs [15], graphene oxide [16], quantum dots (QDs) [17], carbon nanotubes (CNTs) [18] and PLGA [19,20].

Numerous molecules have been introduced for targeting of different vehicles in order to deliver a wide variety of drugs to cancer cells or blood vessels within tumors. These molecules include antibodies, antibody-based fusion proteins, mini bodies, multivalent minibodies, carbohydrates, single-chain dimers and dibodies and aptamers [[21], [22], [23], [24], [25], [26]]. As PLGA is one of the most developed synthetic polymers, various kinds of ligand molecules—such as folic acid (FA) [27], transferrin [28], different kinds of peptides/cyclic peptides, proteins, antibodies and aptamers [[29], [30], [31], [32]]—have been introduced to target PLGA. These targeting ligands could selectively bind to the specific molecules overexpressed by the cancer cells, such as EGFR [31], mannose [33], intercellular cell-adhesion molecule-1 (ICAM-1) [34], human epidermal growth factor

receptor 2 (HER2) [35,36], folate receptors [27,[37], [38], [39], [40]], αVβ3 integrin [[41], [42], [43]], P-glycoprotein [44] and LDL receptor [45].

Aptamers (Apt) are unnatural single-stranded RNA or DNA oligonucleotides (usually 25–90 nucleotide bases) that can fold through intramolecular interactions into complex three-dimensional structures, enabling them to selectively and exclusively bind with relevant biomarkers. Aptamers have been selected as appealing candidates for the fabrication of numerous smart systems, including drug delivery, therapy, diagnosis and bioimaging [46,47]. Many promising features of aptamers—including non-immunogenicity, high stability in a wide range of pH temperatures and organic solvents, low cost, easy synthesis, excellent tissue permeability, ability to characterize and modify, along with high specificity and great binding affinity to binding pockets to various target antigens—allow for their fast application into the clinical environment [48]. Aptamers, as pleiotropic ligands, can be synthesized against unlimited targets through a systematic evolution of ligands by exponential enrichment (SELEX) method [49,50]. Here, we provide complete insight regarding the development of aptamer-grafted PLGA exploited in the delivery of therapeutic agents into tumor cells. The newest and most recent efforts in the field of Apt-conjugated PLGA-based theranostics and stimuli-responsive PLGA as smart DDSs also discussed.

2. Physicochemical characterizations and preparation of PLGA nanoparticles

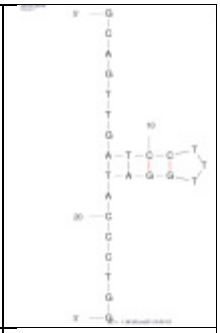
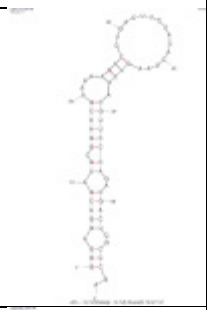
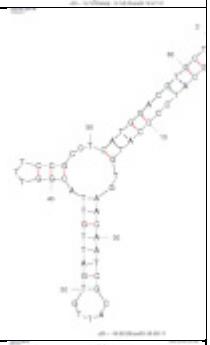
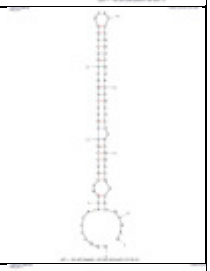
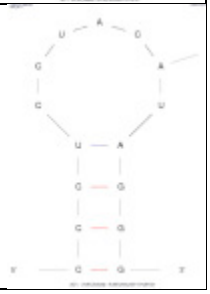
Since the approval of PLGA by the Food and Drug Administration (FDA) in 1969, increasing studies have been performed for the development of various DDSs, both for commercial and research purposes [51]. PLGA is a copolymer of two different monomers—poly lactic acid (PLA) and poly glycolic acid (PGA)—synthesized via ring-opening co-polymerization [52]. It is one of the most popular biodegradable co-polymers because of its remarkable characteristics, such as favorable degradation characteristics, feasibility to design sustained release, option to modify surface features and the possibility to deeply enter into tissues via fine capillaries [5]. Moreover, synthesis approaches of PLGA NPs could be affected by the size of particles, encapsulation efficiency, biological activity, release kinetics and stability of therapeutic agents in the polymer matrix. Double emulsion/solvent evaporation, spray drying, phase separation and nanoprecipitation approaches are the most general preparation methods for synthesis of PLGA [20,[53], [54], [55], [56]].

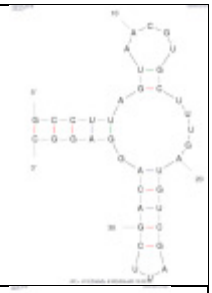
3. Aptamers as targeting agents

Aptamers hold a vital role in drug delivery system development involving active targeting [57,58]. To date, isolation of various aptamers have been accomplished to specifically target receptors on cancer cells, including epithelial cell adhesion molecule (EpCAM) [59], vascular endothelial growth factor (VEGF) [60], platelet-derived growth factor (PDGF) [61], programmed death-ligand 1(PDL1) [62], nuclear factor-kB (NF-kB) [63], prostate-specific membrane antigen (PSMA), nucleolin [64], PTK7 [65] and mucin 1 (MUC1) [66]. Here, the characteristics of the most widely used aptamers for targeting PLGA-based therapeutic systems are reviewed (Table 1).

Table 1. Schematic structure of aptamers using M fold software.

Name of aptamer	**Target**	**Sequence**	**predicted secondary structure by Mfold**

S2.2	MUC1	GCAGTTGATCCTTTGGATACCCTGG	
AS1411	Nucleolin	GGTGGTGGTGGTTGTGGTGGTGGTGG	
A10	PSMA	GGGAGGACGAUGCGGACCGAAAAAGACCUGACUUCUAUACUAAGUCUACG UUCCCAGACGACUCGCCCGA	
TLS11a	MEAR cell	ACA GCA TCC CCA TGT GAA CAA TCG CAT TGT GAT TGT TAC GGT TTC CGC CTC ATG GAC GTG CTG	
EpCAM	EpCAM	GGG ACA CAA UGG ACG UCC GUA GUU CUG GCU GAC UGG UUA CCC GGU CGU ACA GCU CUA ACG GCC GAC AUG AGA G	
A15	CD133	CC CUC CUA CAU AGG G	

CL4	EGFR	GCCUUAGUAACGUGCUUUGAUGUCGAUUCGACAGGAGGC	
A6	HER2	TGG ATGGGGAGATCCGTTGAGTAAGCGGGCGTGTCTCTCTGCCGCCTTGCT ATGGGG	

3.1. Mucin-1-(MUC1) aptamer

MUC1 is an O-glycosylated protein on the surface of most normal secretory epithelial cells. Importantly, it is abnormally overexpressed—known as under-glycosylated form (uMUC1) or tumor-associated MUC1 (TA-MUC1—in a wide range of cancers, which differs from the MUC1 expressed on normal cells during glycosylation, localization, shedding and activities. Until now, several aptamers (including S2.2, S1.1, 5TR1- MUC1, MA3, 5TRG2 and GalNAc3 aptamers) have been selected against exposed epitopes of MUC1 receptors [[66], [67], [68]].

3.2. AS1411 aptamer

AS1411 is a 26-mer DNA aptamer capable of assembling G quadruplex forms that bind to nucleolin, a multifunctional phosphoprotein that is often overexpressed on the cell surface [64,69]. Nucleolin is involved in numerous aspects of cell biology, such as rRNA synthesis, ribosomal synthesis, gene silencing, senescence and cell cycle regulation, regulating cell growth, cell differentiation, apoptosis and DNA replication [70,71]. An AS1411 aptamer against nucleolin is extensively exploited by researchers as a therapeutic option for the treatment of numerous cancers, and this aptamer has even progressed to human clinical trials [72]. The therapeutic benefit of AS1411 aptamer is likely due to the degradation of mRNA of BCL-2 protein and disruption of the nuclear factor-kB (NFκB) signaling that occurs inside cells [73].

3.3. Prostate-specific membrane antigen (PSMA) aptamer

PSMA, a type II membrane protein, is a well-known marker with folate hydrolase activity recognized for its expression on the surface of prostate cancer (PCa) cells. In 2002, for the first time, Lupold and coworkers selected the RNA aptamer detecting PSMA on the surface of prostate tumor cells [74]. Since then, several aptamers against PSMA have been selected—namely, xPSM-A9, xPSM-A10, A10 aptamer, xPSM-A10 e3.2, xPSMA9g, xPSM-A9L and A10–3.2 [75,76].

3.4. TLS11a aptamer

TLS11a aptamer is selected by whole live cell-SELEX against the MEAR cells [77].

3.5. Epithelial cell adhesion molecule (EpCAM) aptamer

EpCAM (CD326 or ESA) is a transmembrane glycoprotein overexpressed in different types of solid tumors and is regarded as a biomarker of cancer stem cells that leads to the regulation of the proto-oncogene c-myc, e-fabp, cyclins A & E and activation of the Wnt signaling pathway [78]. This aptamer was first introduced by Shigdar et al. as a 40-base RNA aptamer—a truncated form of aptamer (19 nt)—with binding affinity of about 55 nM that is successfully internalized following binding to cell surface EpCAM [59].

3.6. CD133 aptamers

CD133 (Prominin-1) is a highly glycosylated, membrane glycoprotein that has been related to the Notch-signaling pathway. It has a role in the differentiation of intestinal epithelium and lymphopoiesis. This glycoprotein has gained the attention of researchers from cancer fields because of its functional role as a marker of cancer stem cells (CSCs) in glioblastomas [79]. Using SELEX, Shigdar et al. identified RNA aptamers (A15, B19) that specifically bind to CD133 protein, which were internalized into CD133 positive cancer cells and penetrated the depth of a three-dimensional tumor sphere [80].

3.7. Epidermal growth factor receptor (EGFR) aptamer

EGFR, a transmembrane glycoprotein (170 kd) is a receptor tyrosine kinase belongs to the ERB-B family. EGFR is activated in many epithelial tumors [81], and multiple aptamers against EGFR have been introduced by different groups—such as E07 RNA aptamer, KDI1 peptide aptamer, CL4 RNA aptamer and TuTu22 DNA aptamer [[82], [83], [84], [85], [86]].

3.8. A6 Aptamer

Human epidermal growth factor receptor 2 (HER2) is characterized by an intrinsic tyrosine kinase property, which is encoded by the ERBB2 gene. Overexpression of HER2 is attributed to aggressive types of breast cancer and is considered as a potential biomarker in cancer immunotherapy [36]. HB5, ECD_Apt1, 15-8 and A6 are some examples of aptamers that specifically bind to HER2-positive breast cancer cells [[87], [88], [89], [90], [91]] (Fig. 1, Fig. 2).

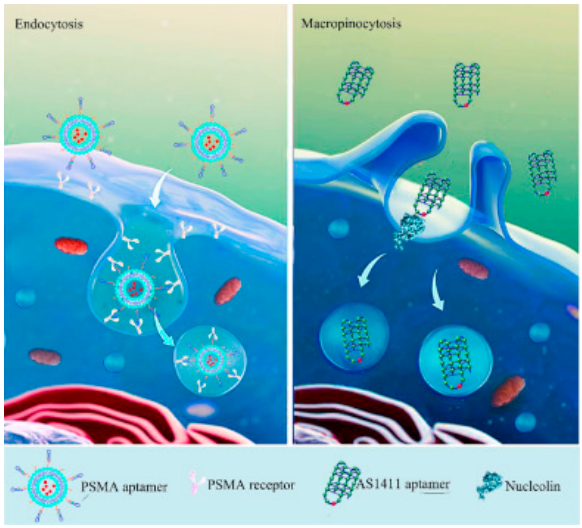


Fig. 1. General scheme of drug targeting delivery to tumors via aptamers.

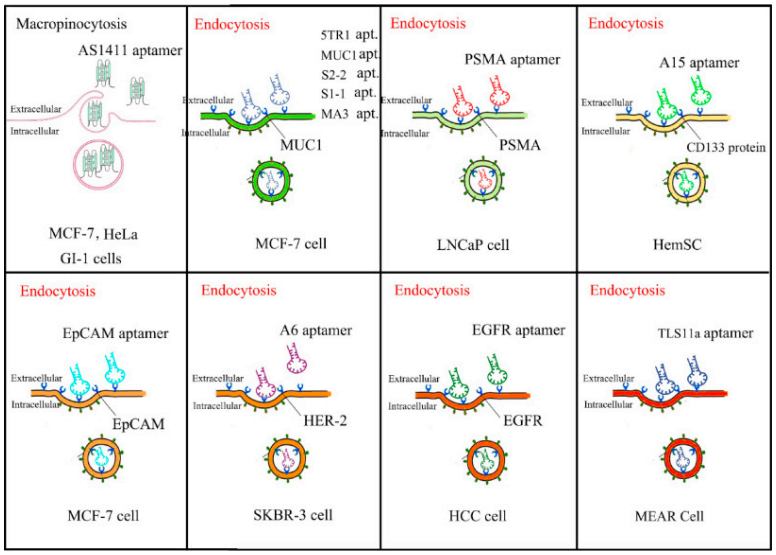


Fig. 2. Schematic illustration of aptamers binding to their specific targets.

4. Aptamer-decorated PLGA for cancer targeted therapy

Many exclusive features of PLGA allow for their use as excellent delivery carriers. These include biodegradability and biocompatibility, ability to encapsulate a wide range of drugs, sustained release, the possibility for controlled and sustained drug delivery, superior stability and great drug loading capacity [53]. Importantly, the PLGA surface can be functionalized with aptamers, as selectively guided targeting ligands, in active drug delivery systems. In this section, aptamer-targeted PLGA NPs for cancer therapy and imaging are reviewed and summarized in Table 2.

Table 2. PLGA-based targeting platforms for the delivery of therapeutic agents.

Targeting Platform	**Aptamer**	**Therapeutic Agents**	**Target Cell Line**	***In Vitro/In Vivo***	**Ref**
PLGA-b-PEG	A10	Cisplatin along Platinum(IV)	LNCaP cells	*In vitro*	[92]
PLGA	AS1411	Paclitaxel	GI-1 cells	*In vitro*	[93]
PLGA-lecithin-PEG	AS1411	Paclitaxel	MCF-7, GI-1 cells	*In vitro*	[94]
PLGA-b-PEG-COOH	PSMA	Docetaxel	LNCaP cells	*In vitro/In vivo*	[95]
PLGA-PEG-COOH	A15	propranolol	HemSCs	*In vitro/In vivo*	[96]
PLGA-PEG-COOH	A15 and CL4	salinomycin	HCC cells	*In vitro/In vivo*	[97]
polydopamine-modified mannitol -PLGA–TPGS	AS1411	Docetaxel	HeLa cell	*In vitro/In vivo*	[98]
PLGA-chitosan	5TR1	Epirubicin	MCF7, C26	*In vitro/In vivo*	[99]
PLGA-PEG-COOH	EpCAM	Doxorubicin	MCF-7	*In vitro*	[100]
magnetic PLGA-PEG	AS1411	Doxorubicin and SPIONs	C6 glioma	*In vitro/In vivo*	[101]
PLGA-PEG-COOH	C2NP	Doxorubicin	L428,Karpass299	*In vitro*	[102]
PLGA-PEG-COOH	TLS11a	Doxorubicin	MEAR	*In vitro*	[103]

PEG- lipid-PLGA	CD133	All-trans retinoic acid	H446, A549	*In vitro/In vivo*	[104]
PLGA-PEG-COOH	HPA(S1.5)	Paclitaxel	MDA-MB-231	*In vitro/In vivo*	[105]
PLGA-PVA	AS1411	Doxorubicin	A549	*In vitro/In vivo*	[106]
PLGA-SS-PEG	EGFR	HHT	A549, NCI–H226	*In vitro/In vivo*	[107]
PLGA-lecithin-PEG	S2.2	Vinorelbine	MCF-7	*In vitro*	[108]

A preclinical study aimed to increase the therapeutic index of cisplatin and minimize its adverse side effects through the synthesis of cisplatin analog platinum (IV) prodrugs. These drugs were loaded in the PLGA-b-PEG NPs using the nano precipitation approach for delivering platinum(IV), a lethal dose of cisplatin, to PSMA-overexpressed prostate cancer cells. A10 aptamer was developed for decorating the surface of the PLGA to facilitate cellular uptake of the Pt(IV)-encapsulated in PLGA by PSMA + LNCaP cells via endocytosis.

Using this new formulation, blood circulation times, drug antitumor efficacy and pharmacokinetics of drugs improved [92]. The first study involving this new formulation was completed by Aravind et al., which assessed the potential of functionalized PLGANPs with aptamer in human glial cancer cells. They used bis (sulfosuccinimidyl) suberate (BS3) as an amine crosslinker, which enabled binding of amine anti-nucleolin aptamer on to the surface of the PLGA. Using this nanoformulation, cellular uptake and apoptotic activities were enhanced in human glial cancer cells [93]. In the same year (2012) in another work carried out by this group, PLGA-lecithin-PEG containing PTX targeted with AS1411 aptamer was developed to actively target the tumor cells that were aggressively expressing nucleolin receptors. Enhanced tumor killing effect, great encapsulation property and superior sustained disease-related agents release were discovered as the main advantages of this targeted lipid–polymer hybrid [94].

In another example, Chen et al. prepared an aptamer-anchored PLGA-b-PEG-COOH for the systemic delivery of docetaxel to prostate cancer cells. In the synthesis procedure, sodium oleate (a small molecule surfactant) was used to reduce the particle size and interfacial tension of PLGA. This diblock copolymer functionalized with PSMA aptamer through EDC and NHS chemistry, and could effectively prompt apoptosis or death of target cells (LNCaP cells) via cell cycle arrest at the G2/M phase.

Another *in vivo* study also indicated the enhancement of antitumor effect and selective cellular uptake of synthesized NPs compared to PLGA lacking aptamer moiety [95]. Poly (ethylene glycol) (PEG) coating was found to bestow long circulating time properties, as well as increase biocompatibility and reduce thrombogenicity on PLGA. It has been proven that the PEGylation of PLGA greatly enhances the passive targeting to tumor cells through retention effects and enhanced permeability. In addition, pegylation of the PLGA surface allows the ability to make them appear invisible to macrophage cells [109,110].

In vitro drug release, entrapment efficiency, cellular uptake, anticancer activity and cytotoxicity were investigated in another study in detail. Subsequently, the *in vivo* study examined pharmacokinetics and drug tissue distribution. Results showed enhancement of anticancer effect and efficient cellular uptake in comparison to PLGA lacking aptamer moiety [95].

Propranolol-loaded PLGA-PEG conjugated with A15 aptamers were used with the aim to reduce the adverse effects of propranolol (a first-line therapy for infantile hemangiomas), as well as the proliferation of hemangioma-derived stem cells (HemSC) both *in vitro* and *in vivo*. In this investigation, propranolol-loaded PLGA was synthesized using the double emulsion method (water/oil/water), and CD133 aptamers were grafted to the

PLGA via an EDC/NHS chemistry. Flow cytometry and HPLC analysis confirmed that targeted PLGA were efficiently bound via a CD133 dependent manner and readily internalized to HemSCs. Propranolol-loaded PLGA have exhibited a sustained release manner up to 8 days, demonstrating the suitability of PLGA as a nano carrier in controlled propranolol liberate. The therapeutic effect of this formulation was observed by lessened hemangioma volume, weight and microvessel density (MVD) [96].

Dual aptamer-functionalized PLGA for promotion delivery of salinomycin to hepatocellular carcinoma (HCC) (CD133+ and CD133-) was introduced by Jiang et al. Dual targeting with A15 aptamer (CD133) and EGFR aptamer (CL4) improved the delivery of salinomycin against both HCC cancer stem cells (CSCs) and a large percent of non-CSCs. Moreover, the excellent antitumor ability in mice bearing HCC xenograft tumors revealed the improved antitumor activity of the system in comparison to other groups [97].

For the first time, a novel and robust nanoparticle, using d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and mannitol PLGA (M-PLGA) loaded with docetaxel, was developed by Xu et al. (2016). Polydopamine (PDA) coated surface of M-PLGA-TPGS polymer and AS1411 aptamer, modified with ten extra T bases at the 3-terminus, were grafted to the surface of DTX-PDA-M-PLGA-TPGS via Michael addition chemistry. In this scenario, increased drug loading capacity and high encapsulation efficiency were achieved by copolymer M-PLGA–TPGS, while significant active targeting was obtained by AS1411 aptamer and exceptional long-term compatibility was attained by incorporation of TPGS [98].

PLGA NPs decorated with 5TR1 aptamer and surface functionalization combined with chitosan has shown increased significant tumor growth inhibition in BALB/c mice bearing C26 cells [99]. The overexpression of EpCAM has been capitalized for targeting of many drug-aptamer-PLGA. For instance, PEG-PLGA nanopolymerosomes (<120 nm) were synthesized using a pH gradient method. EpCAM aptamers were covalently grafted on the surface of polymerosomes loaded with DOX. This targeted system successfully could inhibit the growth of human breast adenocarcinoma *in vitro* [100].

Mosafer et al. (2017) synthesized AS1411 aptamer-PLGA as a promising functionalized delivery for glioma cancer therapy. Superparamagnetic iron oxide nanocrystals (SPIONs) and DOX were encapsulated in the PLGA using modified multiple emulsion solvent evaporation technique. The authors achieved enhancement of the therapeutic effect and internalization of AS1411 aptamer-PLGA-DOX for C6 glioma compared to the L929 cell line (no target cell) [101]. (see Fig. 3).

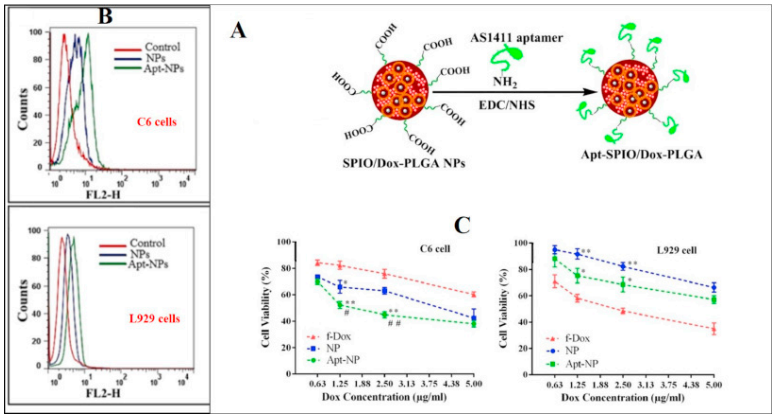


Fig. 3. (A) Schematic illustration of aptamer-anchored SPIONs-PLGA NPs for site-specific DOX delivery. (B) Flow cytometry assay for specific cell binding characterization. (C) Cytotoxicity of DOX on C6 cells (target cells) in comparison to L929 cells (non-target). Reproduced with permission from Ref. [101].

In a similar study, PLGA nanoparticles loaded with DOX were decorated with C2NP aptamer to target anaplastic large cell lymphoma cells over expressed CD30. *In vitro*, this targeted nanosystem showed high uptake by the K299 cell, leading to growth inhibition and induction of apoptosis in these tumor cells up to 61% [102]. Grafting amine-modified TLS11a aptamer to PLGA-PEG resulted in effect binding to hepatocellular carcinoma cells (MEAR cell line). A higher therapeutic effect was observed when loaded with DOX compared with PLGA, without the targeting aptamer [103].

Lipid-PLGA hybrid NPs, composed of a polymeric core as well as a lipid shell, exhibited great potential as a delivery system for the treatment of some cancers. In this context, Zhang et al. (2018) formulated all-trans retinoic acid (RA) loaded lipid-PLGA nanoparticles targeted with thiolated CD133 aptamers (RA-LPNPs-CD133) against CD133+ lung cancer initiating cells. In this nanoformulation, a lipid film was composed of DSPE-PEG-Mal and cholesterol in a 57:3:40 M ratio. Cholesterol improved lipid bilayer stability and diminished the permeability of liposomal bilayers, resulting in reduced leakage of loaded drugs during circulation in the body. Additionally, cholesterol minimized side effects and enhanced the bioavailability of drugs [111]. RA-LPNPs-CD133 showed sustained release of RA during the 144 h and exhibited higher inhibitory properties toward CD133+ lung cancer using the CD133 aptamers compared to non-targeted PLGA and RA [104].

In another study, PLGA functionalized with HPA aptamer (S1.5) was designed to specifically release PTX in HPA-overexpressed MDA-MB-231 cells. Targeted PLGA showed high uptake, superior anti-angiogenesis and noticeable eradication properties of triple-negative breast cancer cells through HPA-related signaling pathways. The *in vivo* anti-tumor studies revealed that the Apt (S1.5)-PTX-PLGA could reduce tumor sizes over the control group [105]. More recently, Saravanakumar et al. (2019) synthesized and tested a novel targeted pH-responsive system against lung cancer cells (A549). In this investigation, PLGA-DOX was stabilized with poly (N-vinylpyrrolidone), which was then decorated with pH-dependent AS1411 aptamer to trigger drug release and cancer cell death. Besides, the authors demonstrated that the APT-DOX-PLGA-PVP NPs induced apoptosis signaling pathways via the activation of the apoptosis-related proteins [106].

Zhang et al. developed a novel targeted PLGA-based redox-responsive system for delivery of homoharringtonine (HHT) as an effective anticancer agent for the treatment of lung cancer both *in vitro* and *in vivo*. In this study, the PLGA contains a redox-cleavable disulfide linker, which can be broken by the high glutathione (GSH) level in lung cancer cells, leading to the release of homoharringtonine (HHT) into the cytoplasm of tumor cells. They revealed the great potential of this targeted redox-responsive system in inhibition of tumor growth with high efficacy, as well as its stimulation of apoptosis of lung cancer cells through the mitochondria pathway in lung cancer [107].

Liu et al. incorporated S2.2 aptamer to lipid PLGA to design a nanoformulation to overcome the drawbacks associated with direct administration of vinorelbine (VRL), such as venous irritation, phlebitis, poor solubility in water and toxicity to normal cells. The incorporation of a lipid shell in this nanoformulation could provide a sustained drug release profile due to the lipid shell hindering the penetration of water molecules into the core of the NPs, thereby minimizing core degradation. Therefore, it can provide sustained release manner over a longer period. Moreover, the S2.2 aptamer has improved the efficacy of the VRL by more than five-fold [108].

5. Aptamer-targeted PLGA NPs for nucleic acid delivery

Nucleic acids may either stimulate gene expression by the addition of an exogenous gene (cDNA) or silence expression of aberrant or pathologic genes (known as RNAi mediators). Recently, research has demonstrated that PLGA is an excellent carrier for nucleic acid-based cargos—such as siRNA, miRNA and plasmid DNA—with respect to transfection efficiency [56,[112], [113], [114]]. Generally, nucleic acids can be encapsulated into the

core of PLGA, or adsorbed on the cationic PLGA surface through electrostatic interactions [115,116]. In different studies, aptamer-targeted PLGA NPs have been used for gene delivery, which are summarized in Table 3.

Table 3. PLGA-based targeting platforms for the delivery of genes.

Targeting Platform	**Aptamer**	**Therapeutic Agents**	**Gene**	**Target Cell Line**	***In Vitro/In Vivo***	**Ref**
PβAEC32 and PLGA	MUC1	Epirubicin	antiMiR-21	MCF7, C26	*In vitro/In vivo*	[117]
Liposome PLGA-Mal-PEG	A6	–	P-gp siRNA	SKBR-3, 4T1-R	*In vitro*	[118]
PLGA-PEG-COOH	AS1411	cisplatin	antiMiR-21	A2780	*In vitro*	[119]
PLGA-PEG-COOH	A10	–	TFO	LNCaP	*In vitro/In vivo*	[120]

A targeted nanosystem composed of two polymers—PβAEC32 and PLGA—was used to co-deliver MiR-21, an oncomiR overexpressed in different types of cancer, and epirubicin (EPI). Anti-miR-21 inhibits the expression of miR-21, resulting in enhanced chemotherapy. Briefly, in this platform, MiR-21 and PβAE polymer formed a polyplex in the inner core. Simultaneously, EPI and polyplex were then added to the PLGA solution. Subsequently, PLGA was deposited electrostatically on the surface of the polyplex, providing the entrapment of the EPI. MUC1 aptamer was then coated covalently on the surface of PLGA. This nanocomplex exhibited remarkable cytotoxicity for the MCF7 and C26 cells in comparison with CHO cells (MUC1 negative). In addition, a synergistic and marked therapeutic effect of EPI and antimir-21 was observed in preventing the tumor growth in C26 cells-bearing mice in comparison to mice cured with free EPI or PLGA loaded only with EPI or antimir-21(117).

The main advantage of this formulation is incorporation of PβAE (cationic polymer) to enable the loading of the negatively charged gene inside PLGA and improving gene payload encapsulation efficiency [121]. To overcome multi-drug resistance (MDR), it was suggested that hybrid nanoparticles (cationic lipids and PLGA-b-PEG) guided by A6 aptamer deliver p-gp siRNA into the metastatic breast cancer cells (Her-2+). The lipid bilayer prevented nanoparticle aggregation and improved RNAi transfect efficiency. Furthermore, cationic moieties also improved interaction between cell membranes (negative charge) and nanoparticles (positive charge) and/or prompted endosomal escape [122,123].

Aptamer A6, which has an affinity for HER2 receptors on breast cancer cells, conjugated to the hybrid nanoparticle through thiol-maleimide chemistry. Using this targeted formulation, a marked increase in siRNA delivery and major silencing activity in breast cancer cells was detected [118]. PLGA-cisplatin in combination with PLGA-anti-miRNA-21 targeted with AS1411 was administered as a potential therapy for the treatment of the ovarian cancers resistance to cisplatin. Due to the ability of anti-miR-21 to ameliorate cisplatin resistance, ovarian cancer cells were pretreated with AS1411 -PLGA-anti-miRNA-21, then miR-21-inhibited ovarian cancer cells were exposed to the AS1411 aptamer–cisplatin–PLGA. Using this combination therapy, the incidence of the late apoptosis improved in ovarian cancer cells resistance to cisplatin [119].

Jiao et al. designed a new nanosystem for gene therapy of prostate cancer. PLG-PEG copolymer loaded with TFO (triplex forming oligonucleotide, androgen receptor (AR) siRNA) using the water-in-oil-in-water (w1/o/w2) further functioned with A10 aptamer. An *in vitro* study revealed increased cellular toxicity in A10 aptamer-PLG NPs. The LNCaP xenograft tumor regression decreased, in comparison to the non-targeted system. On the other hand, A10 aptamer-PLG loaded with TFO could silence AR gene expression in prostate cancer cells [120].

6. Aptamer-conjugated PLGA-based theranostics

The combination of therapeutic and imaging reagents in a single multifunction nanosystem is considered as theranostics. PLGA NPs are employed as suitable theranostic tools that can be easily modified by the addition of imaging agents—such as quantum dots, MRI contrast agents, fluorescent dyes, SPION or radiotracers—during the particle synthesis process. Numerous studies have focused on the development of targeted PLGA for simultaneous active targeting and imaging in several types of cancers. In this context, superparamagnetic iron oxide nanoparticles (SPIONs) and DOX were entrapped in PLGA-PEG-COOH copolymer and further decorated with AS1411 aptamer, suggesting that these targeted magnetic composite nanoparticles might be employed as controlled delivery of anticancer drugs. Higher tumor targeting was detected by MRI for the PLGA-PEG-COOH coated with the AS1411 aptamer compared to unconjugated nanoparticles. In addition, both tumor growth inhibition and sustained survival of mice (bearing C26 colon carcinoma xenografts) were significantly greater in the targeted PLGA-PEG-COOH group [124] (see Fig. 4).

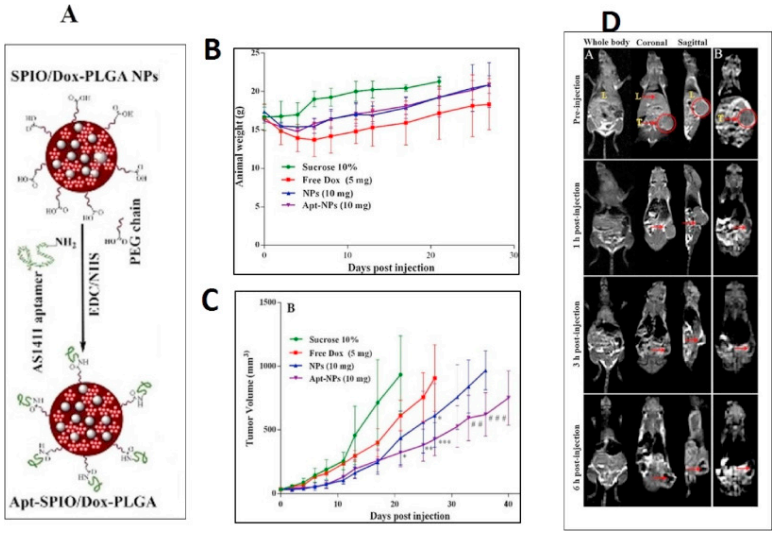


Fig. 4. (A) Schematic representation of multifunctional theranostic Apt-NPs for cancer therapy and imaging. (B) Body weight of C26 tumor-bearing mice after treatments with free DOX solution, NPs and Apt-NPs. (C) Efficiency of *in vivo* tumor suppression against C26 colon carcinoma. (D) T1-weighted MR images of female BALB/c mice bearing C26 colon carcinoma in different time points before and after injection of NPs. Reproduced with permission from Ref [124].

In another work, in order to improve sensitivity and specificity in prostate cancer diagnosis, Wu and colleagues constructed gas-filled nanobubbles (NBs) from PLGA encapsulated paclitaxel (PTX), which was further modified with anti-PSMA A10–3.2 aptamer as the contrast agent for ultrasonography and drug carrier. Within this scenario, PTX-A10-3.2-PLGA NBs combined with the US represented an effective approach for targeted delivery of PTX to prostate cancer, without considerable side effects. In the US images compared with the control group, the distribution of contrast agent echo signals were strength, and augmentation of contrast were detected in mice treated with A10-3.2-PLGA NBs. The A10-3.2-PLGA NBs exhibited strong signal intensity, peaking around 25 dB (PLGA NBs˷ 15.4 dB) [125].

For theranostic purposes, a nanocomposite of SPION and curcumin co-encapsulated in PLGA nanoparticles were designed as a photothermal treatment and multimodal imaging agents of pancreatic cancer cells with overexpressed nucleolin receptors (PANC-1 and MIA PaCa-2 cancer cell lines). The AS1411 aptamer was joined to the nanocomposite (via the EDC/NHS reaction) to enable targeted treatment of pancreatic tumor cells. Efficacy of the nanocomposite as spin–spin (T2) contrast agent was performed by relaxometry studies and

phantom MRI. *In vitro* studies in cell cultures have shown cytotoxicity and photothermal ablation of cancer cells. As a result of aptamer-curcumin-SPION loaded PLGA, this has suggested that these nanocomposites are promising carriers for efficient therapy of pancreatic cancer [126]. The theranostic abilities of nutlin-3, a loaded PLGA decorated by EpCAM aptamer, was investigated in MCF-7and ZR751 (human breast cancer) and SKOV3 human ovarian cancer cell lines. In this multifunctional structure, quantum dots were used as the imaging agent to monitor inhibition of both 3D spheroid tumor and 2D monolayer model [127].

Conclusion and future prospective functional nucleic acids for cancer theranostics

Aptamers are exceptional targeting ligands with similar, or even greater, binding affinity in comparison to antibodies. They can discriminate cancer cells from healthy cells with high sensitivity and specificity. Incorporation of aptamers within PLGA could result in applicable platforms with diagnosis, therapeutic capability with high therapeutic efficiency and reduced side effects in tumor therapy. On the other hand, PLGA also can improve the efficacy of treatments, along with the pharmacokinetic and pharmacodynamic profiles of drugs. In the current state, many models have proven their potential benefit regarding use of PLGA and aptamer in the management of various cancers (Table 2, Table 3). These examples noticeably illustrate the promise of PLGA-aptamer for new treatments in the near future.

Authorship contribution statement

Azarmidokht Shamshiri: Writing - original draft, Writing - review & editing.
Maryam Hashemi: Writing - review & editing.
Majid Saeedi: Writing - review & editing.
Rezvan Yazdian-Robati: Conceptualization, Writing - review & editing.
Lobat Tayebi : Conceptualization, Writing - review & editing.

Declaration of competing interest

There is no conflict of interest regarding this article.

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