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# Oligonucleotide-conjugated nanoparticles for targeted drug delivery via scavenger receptors class A: An *in vitro* assessment for proof-of-concept

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ABSTRACT

Spherical nucleic acid gold nanoparticles represent a unique nanotechnology in which the spherical arrangement of oligonucleotides enables the nanoparticles to be efficiently internalized into cells expressing scavenger receptors class A (SR-A). Herein, we seek to replace the gold core with a biodegradable polymeric construct and explore their potential applications in targeted drug delivery.

Oligonucleotide-conjugated poly(ethylene glycol)-block-poly(ε-caprolactone) was synthesized and characterized by $^{1}$H NMR and gel electrophoresis. This polymer was applied to fabricate micellar nanoparticles (OLN-NPs) by an anti-solvent method. These nanoparticles have a mean particle size about 58.1 nm with a narrow size distribution (PDI $<0.2$) and they were also non-cytotoxic. Relative to non-targeted NPs, OLN-NPs exhibited substantially better uptake (3.94×) in a mouse endothelial cell line (C166), attributing to lipid-raft-mediated endocytosis via SR-A.

To explore the potential applications of OLN-NPs as drug carriers, paclitaxel, a poorly soluble anti-angiogenic compound, was selected as the model. OLN-NPs increased the solubility of paclitaxel by at least 300×. The boosted drug solubility in conjunction with improved cellular uptake translated into enhanced *in vitro* efficacy in the inhibition of angiogenesis. In conclusions, OLN-NPs show considerable promise in targeted drug delivery and their potential applications should be further investigated.

## 1. Introduction

Recent advances in nanotechnology have ignited the transformation of research in drug delivery and disease diagnosis. Of particular merit is spherical nucleic acid gold nanoparticles (SNA-AuNPs) which were introduced by Mirkin's lab in 1996 (Mirkin et al., 1996). Contrary to linear nucleic acids which cannot penetrate cells, the unique spherical arrangement of oligonucleotides enables the NPs to be efficiently internalized into cells expressing SR-AI/AII (also known as SR-A, MRS-A, Scara1, Scvr and CD204; In this paper, we refer SR-AI/AII as SR-A) and resist degradation from nucleases (Choi et al., 2013). SNA-AuNPs also exhibit gene transfection and regulation without the need for a transfecting agent (Zhang et al., 2015). Since then, this nanotechnology has been explored in different applications including *in vitro* detection (Nam et al., 2004), intracellular assays (Mirkin, 2010), cell transfection (Rink et al., 2010), therapeutic and gene regulation (Jensen et al., 2013; Zhang et al., 2015). It has been demonstrated that SNA-AuNPs were uptaken into cells by lipid-raft-mediated (through CAV1) endocytosis via SR-A (Choi et al., 2013). SR-A are Type II membrane proteins of approximately 400–500 residues with an N-terminus comprising a short cytoplasmic domain followed by a single transmembrane region and a large extracellular domain that mediates ligand recognition (Zani et al., 2015). They are expressed primarily on macrophages, dendritic, epithelial and endothelial cells as a first-line host defense. These cells are potential targets for the treatment of various diseases, for instance, cancer, diabetes, infectious diseases, and sight-threatening eye diseases (Liu et al., 2014).

Despite the widespread applications of SNA-AuNPs in different areas, the utilization of this technology in drug delivery has been seldom explored (Zhang et al., 2011). The major challenge stems from the complexity of drug conjugation onto the nanoparticles. Drug molecules must be first "chemically activated" via the introduction of functional groups for attachment. It is very difficult to generalize this process because of the huge diversity of the chemical structures of drug

*Abbreviations:* PEG-NPs, Nanoparticles of PEG-b-PCL; OLN-NPs, Nanoparticles of OLN-PEG-b-PCL

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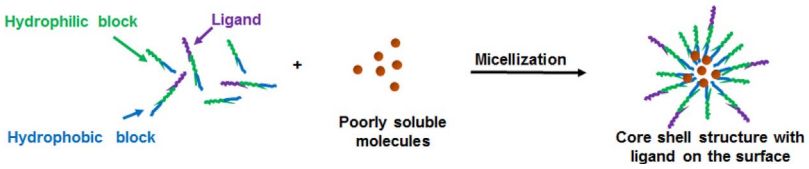


**Fig. 1.** Encapsulation of poorly soluble drugs into polymeric nanoparticles.

molecules. In other words, this "chemical activation" process must be tailored for each drug molecule. As a result, the application of a biodegradable polymeric core which enables drug solubilization via physical entrapment is highly desirable. Indeed, in addition to inefficient intracellular delivery, poor drug solubility is another major stumbling block in current drug delivery research.

In light of this, herein, we report the use of oligonucleotide-conjugated poly(ethylene glycol)-block-poly(ε-caprolactone) nanoparticles (OLN-NPs) as carriers for targeted drug delivery to cells expressing SR-A. Poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-b-PCL) was selected as the skeleton of our nanotechnology design, attributing to its superb drug solubilizing capacity, biocompatibility, and biodegradability. This amphiphilic block copolymer consists of both hydrophilic and hydrophobic segments, which undergo self-assembly to form micellar nanoparticles (NPs) (Fig. 1) (Croy and Kwon, 2006; Letchford and Burt, 2007). The unique core-shell structure increases the solubility of poorly soluble drugs by incorporating them into the hydrophobic cores and the outer hydrophilic shells prolong the circulation time of the NPs inside the body (Kedar et al., 2010; Liu et al., 2007). Although these highly tunable nanocarriers (Soleymani Abyaneh et al., 2015) offer many advantages, their passive targeting may result in inefficient intracellular drug delivery, negatively impacting the treatment efficacy and/or safety profiles of drugs (Wilhelm et al., 2016). In this research, we endowed PEG-NPs with the specific targeting ability of OLN. T30, an oligonucleotide with 30 repeating units of thymidine which was previously applied to improve the cellular uptake of AuNPs (Yang et al., 2016), was employed as the targeting ligand for our nanotechnology. The unique combination of OLN and PEG-b-PCL can potentially overcome the major challenges of drug delivery: poor drug solubility and lack of targeting/inefficient cellular uptake.

Aimed at demonstrating the feasibility of our nanotechnology for targeted drug delivery to cells expressing SR-A and exploring its potential applications, in this study, we employed coumarin 6 and paclitaxel as model compounds. Coumarin 6 is a poorly soluble fluorescent dye which would be used to elucidate the cellular uptake mechanisms of the NPs. Paclitaxel is a poorly soluble anti-angiogenic agent which would be used to assess the potential applications of OLN-NPs as an anti-angiogenic therapy. Accordingly, our objectives were: (1) to synthesize OLN-PEG-b-PCL and fabricate NPs from this polymer; (2) to investigate the cellular uptake and uptake mechanisms of these NPs. To further prove our concept and explore the potential applications, we encapsulated paclitaxel into OLN-NPs and assessed their *in vitro* efficacy in the inhibition of angiogenesis. This NP-based anti-angiogenic therapy can be potentially applied locally for the treatment of sight-threatening eye diseases such as corneal angiogenesis, aged-related macular degeneration, diabetic retinopathy, etc. Indeed, up-regulation of SR-A was observed in an animal model of age-related macular degeneration (Jawad et al., 2013). Alternatively, they can be administered systemically for the treatment of other angiogenesis-related diseases such as cancer. Indeed, our NPs are not limited to anti-angiogenic therapy, and they can serve as a carrier to deliver different drug molecules with diverse physicochemical properties to cells expressing SR-A for various diseases (Liu et al., 2014).

## 2. Materials and methods

### 2.1. Materials

Polyethylene glycol (average $M_n$ = 3350), triethylamine, tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl), coumarin 6, uranyl acetate, chlorpromazine, methyl-β-cyclodextrin and trisaminomethane (Tris) were purchased from Sigma-Aldrich (St. Louis, USA). Caprolactone and 4-dimethylaminopyridine were obtained from J & K Scientific (China). 1-pentanol, succinic anhydride, oxalyl chloride and acryloyl chloride were procured from TCI (Tokyo, Japan). Paclitaxel was purchased from LC Laboratories (Massachusetts, USA). Fucoidan was acquired from Santa Cruz Biotechnology (California, USA). Unless otherwise stated, all materials were purchased from commercial sources and used as received without further purification.

### 2.2. General synthesis consideration

All of our synthesis experiments were conducted using dried glassware and anhydrous solvents under a nitrogen atmosphere unless stated otherwise. AR grade tetrahydrofuran, toluene, *N, N*-dimethylformamide and dichloromethane were dried using a 4 Å molecular sieve. HPLC grade acetonitrile was distilled from calcium hydride. Water was purified from Direct-Q® UV Water Purification System (EMD Millipore, USA). Unless otherwise stated, type 1 water was used in the preparation of all aqueous solutions.

### 2.3. Synthesis of T30 oligonucleotides

Thiolated DNA oligonucleotides with 30 repeating units of thymidine (T30-SH) were synthesized with an automated synthesizer (Oligo 800 Azco Biotech) by using standard solid-phase synthesis and reagents (Yang et al., 2016). The synthesized oligonucleotides were purified by using an Agilent 1260 high-performance liquid chromatography (Santa Clara, California, USA) with a Microsorb C18 column (Varian).

### 2.4. Synthesis of hydroxyl-functionalized PEG-b-PCL

Hydroxyl-functionalized PEG-b-PCL (HO-PEG-b-PCL) was synthesized according to a method reported previously using 1-pentanol as an initiator (Ji et al., 2009). The average $M_n$ of the PEG used was 3350 and the $M_n$ of the PCL segment in the PEG-b-PCL was determined by $^1$H NMR (Supplementary materials, Fig. 1).

### 2.5. Synthesis of acrylate functionalized PEG-b-PCL

Triethylamine (1 mmol, 10 equiv.) and acryloyl chloride (1 mmol, 10 equiv.) were added to a solution of HO-PEG-b-PCL (0.1 mmol, 1 equiv.) in dichloromethane (10 mL) at 0 °C. The reaction mixture was subsequently stirred at room temperature for 16 h. It was then concentrated *in vacuo*, and the residue was dissolved in a minimum amount of dichloromethane. Next, the product was precipitated and repeatedly washed with methanol. Acrylate functionality was confirmed by the presence of UV absorbance at 254 nm. Methyl acrylate was used as a UV standard for the determination of the% functionalization of the PEG-b-PCL. In general, at least 80% functionalization was observed.