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Leading Opinion

A strategy for precision engineering of nanoparticles of biodegradable copolymers for quantitative control of targeted drug delivery☆

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ABSTRACT

Research on quantitative control of targeting effect for the drug delivery system of ligand-conjugated nanoparticles of biodegradable polymers is at the cutting edge in the design of drug delivery device. In this work, we developed a post-conjugation strategy, which makes the ligand conjugation after the preparation of the drug-loaded nanoparticles of two copolymers blend. We synthesized the PLGA-PEG copolymer with PEG functioning as the linker molecule needed for herceptin conjugation. Docetaxel-loaded nanoparticles of the PLGA-PEG/PLGA copolymer blend were prepared by the nanoprecipitation method. Anti-HER2 antibody (heceptin), which targets the breast cancer cells of HER2 receptor over-expression, was conjugated on the drug-loaded PLGA-PEG/PLGA nanoparticles for sustained, controlled and targeted delivery of docetaxel. We demonstrated that the targeting effect can be quantitatively controlled by two ways, *i.e.* (1) adjusting the copolymer blend ratio of the nanoparticle matrix, which showed within the range of 20% PLGA/PEG in the copolymer blend a linear relation with the ligand density on the nanoparticle surface, and (2) adjusting the herceptin feed molar ratio to NH2 in the linker molecules appearing on the nanoparticle surface, which also showed a linear relation. Compared with the pre-conjugation strategy developed recently in the literature, in which the ligand was firstly conjugated onto the PLGA-PEG copolymer before preparation of the nanoparticles of the PLGA-PEG/PLGA copolymer blend, the post-conjugation strategy provides more efficient use of the ligand and protects its bioactivity in the nanoparticle preparation process, thus resulting in much better performance in drug targeting, which was assessed *in vitro* with SK-BR-3 breast cancer cells of HER2 receptor overexpression and MCF7 breast cancer cells of HER2 receptors moderate expression.

1. Introduction

Drug delivery device (DDD) of controlled and targeted function can ideally deliver high dose of the therapeutic agent specifically to the diseased cells with the healthy cells less interfered, thus resulting desired pharmacokinetics and biodistribution for higher therapeutic effect and fewer side effect. Such kind of idea for a well-designed administration of therapeutics could be traced back to Paul Ehrlich, the 1906 Nobel Prize Medicine Laureate, and his concept of magic bullets. Since then it has been arousing continuous interest in developing various advanced targeting strategies, among which ligand-conjugated nanoparticles (including soft nanoparticles such as micelles and liposomes and solid nanoparticles such as those of biodegradable polymers) may be the most prospective among various targeted DDD [1–6]. Polymeric nanoparticles (NPs) are able to dissolve hydrophobic drugs in polymeric matrix, solving the drug solubility problem as well as possess the advantages such as high stability, efficient drug load, sustained drug release, enhanced circulation time in bloodstream, and active targeting space for cancer cells [7,8]. A good example is the polymeric nanoparticle formulation of docetaxel, a potent anticancer drug approved by FDA for the treatment on a wide spectrum of cancers [9], which has aroused high attraction recently [10–13]. Among those, targeted formulations are believed to be capable of distinguishing the cancer cells from normal cells as well as accumulating in the cancer cells.

☆ *Editor's Note*: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

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Targeted drug delivery is of necessary importance to achieve "on-site" delivery. Passive targeting can be realized by the enhanced permeation and retention (EPR) effect of the leaky vessels of tumors which allow the drug carrier nanoparticles of appropriate size and surface properties accumulated in the tumor. Active targeting presents a more promising approach to the purpose, which can be realized by conjugating molecular probes or ligands onto the surface of the nanocarriers, providing drug delivery systems for reaching and penetrating into the malignant cells which are of overexpression of the corresponding receptors on their membrane, and then releasing the encapsulated therapeutics in the diseased cells in a controlled and sustained manner [14]. The candidates of various targeting ligands include small molecules [15], peptides [16], antibodies [17] and affibodies [18]. Among them herceptin (or Herceptin®, the clinical formulation of Trastuzumab invented by Genentech), the first humanized antibody approved by FDA for the treatment of human epidermal growth factor receptor type 2 (HER2)-positive metastatic breast cancers, has widely appeared in recent studies [19,20]. It is known that HER2 overexpresses in 25–30% invasive breast cancers. Herceptin as a promising therapy for advanced breast cancers is able to specifically bind to the extracellular juxtamembrane domain of HER2 and inhibits the proliferation and survival of HER2-dependent tumor cells, which is an excellent strategy for drug targeting [21,22]. Herceptin is also managed to efficiently internalize into the cells through the receptor-mediated endocytosis (RME) even when conjugated with a wide variety of molecules [23,24]. Herceptin and its conjugates with toxins or nanoparticle formulations were widely used for selective delivery of anticancer agents to cells with positive HER2 receptors [25–29].

Tailoring of the functional nanocarriers depends on the selection of matrix materials as well as functionalization of surface property. A good example in the literature is to use poly (lactide-co-glycolide) (PLGA), one of the FDA approved biodegradable polymers widely applied in the drug delivery realm, as the core of the NPs and poly (ethylene glycol) (PEG), a water soluble polymer widely used to enhance biocompatibility and circulation half-life as well as to make nanocarriers escape from being recognized and eliminated by the reticuloendothelial system (RES), to facilitate functionalization of the nanoparticle surface by antibody conjugation. The PEG layer coated over the PLGA core thus makes the NPs stealth property which is the basic property to achieve passive targeting purpose. In addition, the functional PEG chains provide the reaction site for antibody decoration on the NPs. Conjugation of PEG with PLGA is an effective method to alter the property of the NPs' surface while keeping the unity of polymeric core [30,31]. Another example is to use a copolymer between poly(lactic acid) and D-alpha-tocopheryl polyethylene glycol 1000 succinate (PLA-TPGS), which promotes better performance for the nanoparticle drug delivery system, as the polymeric core and the carboxyl group terminated TPGS (TPGS-COOH) as the linker molecules for ligand (for example herceptin or folic acid) conjugation [27,32]. All these examples showed *in vitro* and *in vivo* the qualitative targeting effects of the anticancer drugs formulated in the nanoparticles of the copolymer blend for cancer treatment in comparison with the corresponding nanoparticle system of no targeting effects. There have been only a few reports in the literature that demonstrates a quantitative effect for drug targeting.

Ligand conjugation is a self-assembly process in which no direct management available to control successful conjugation between the ligand and the polymeric nanoparticles, which makes it difficult for any quantitative control of the targeting effect to meet the treatment needs to be realized. Precision engineering of the nanoparticles for targeted drug delivery means to develop a practical strategy that can control the quantity, *i.e.* the surface density, of the targeting ligand on the NP surface. Although little of such work could be found so far from the literature, this task represents an important aspect at the cutting edge in the design of DDD for drug targeting. To certain extent, the ligand density on the surface of drug delivery systems is believed as one of the essential biophysicochemical properties as important as size, shape, charge, and surface hydrophilicity of the nanoparticle drug delivery system [3]. Moreover, the surface density of conjugated ligand is an external indicator for the surface chemistry of NPs which is one of the important properties of NPs affecting the safety issues of NPs in biomedical applications [33]. The control of the ligand density on the NPs surface exerts the carriers in a more precise manner as well as facilitates the balance between tissue penetration and cellular uptake, resulting in optimal therapeutic efficacy [3]. Recently, there are two strategies developed for quantitative control of the targeting effects by adjusting the ligand density on the nanoparticle surface through varying the copolymer ratio in the nanoparticle matrix consisting of two copolymers with one of a linker molecule for ligand conjugation, which we call the pre-conjugation and the post-conjugation respectively, *i.e.* to conjugate the ligand to the linker molecule before or after the nanoparticle formulation. For the pre-conjugation strategy, one copolymer such as PLGA-PEG of the nanoparticle matrix was firstly conjugated with targeting ligand say A10 aptamer, the NPs was then prepared say by the nanoprecipitation method [34]. The disadvantage of such a strategy is clear. Only part of the ligand would appear on the nanoparticle surface with large number of the ligand wasted within the polymeric matrix, leading to insufficient quantity of the targeting moieties on the nanoparticle surface. Moreover, the ligand distribution on the NP surface would not be uniform, resulting in irregular distribution of the ligand among the various nanoparticles since not all of the polymeric macromolecules of the linker molecule can be made use of in the NP formulation process. Moreover, the ligand molecules are usually fragile biomolecules of complex conformation that may be inactivated in the organic solvent used in the NP preparation process, thus weakening the targeting effects. This strategy, therefore, is not as desired to precisely control the targeting effect. For the post-conjugation strategy, instead, the drug-loaded nanoparticles were firstly prepared with the two copolymer blend such PLA-TPGS and TPGS-COOH. The nanoparticles were then functionalized by the ligand such as herceptin or folic acid [27,32,35]. The advantages of such a post-conjugation strategy thus overcome the weakness of the pre-conjugation strategy, which used the ligand much more efficiently and protects its bioactivity, thus resulting much more effective targeting effects.

In the present study, we intend to show the feasibility of the post-conjugation strategy for quantitative control of the targeting effect by controlling the ligand density on the nanoparticle surface and to demonstrate its impact on the cellular uptake efficiency and cytotoxicity. To our knowledge, there is no report so far in the literature on the formulation of docetaxel, one of the most effective anticancer drugs of the highest annual sale in the world market, by herceptin-conjugated nanoparticles of the PLGA-PEG/PLGA copolymer blend although a pre-conjugation strategy for targeted delivery of docetaxel by the A10 aptamer-conjugated nanoparticles was recently developed from Langer's group at MIT [34]. For the first time in the literature, we demonstrate that the surface density of the ligand molecules can be precisely controlled by adjusting the blend ratio of the two copolymers in the polymeric matrix. In fact, a linear relation between these two parameters can be achieved within certain range of the copolymer blend ratio. We synthesized PLGA-PEG block copolymer, which was then mixed with PLGA at a designated blend ratio to prepare the docetaxel-loaded NPs of the PLGA-PEG/PLGA copolymer blend. The distal primary amine groups on the PEG chain were utilized to conjugate the carboxylic groups