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Quantitative control of active targeting of nanocarriers to tumor cells through optimization of folate ligand density

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ABSTRACT

The active targeting delivery system has been widely studied in cancer therapy by utilizing folate (FA) ligands to generate specific interaction between nanocarriers and folate receptors (FRs) on tumor cell. However, there is little work that has been published to investigate the influence of the definite density of the FA ligands on the active targeting of nanocarriers. In this study, we have combined magnetic-guided iron oxide nanoparticles with FA ligands, adjusted the FA ligand density and then studied the resulting effects on the active targeting ability of this dual-targeting drug delivery system to tumor cells. We have also optimized the FA ligand density of the drug delivery system for their active targeting to FR-overexpressing tumor cells *in vitro*. Prussian blue staining, semi-thin section of cells observed with transmission electron microscopy (TEM) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES) have shown that the optimal FA density is from 2.3×10^{18} to 2.5×10^{18} per gram nanoparticles ($(g \cdot NPs)^{-1}$). We have further tried to qualitatively and quantitatively control the active targeting and delivering of drugs to tumors on 4T1-bearing BALB/c mice. As expected, the *in vivo* experimental results have also demonstrated that the FA density of the magnetic nanoparticles (MNPs) could be optimized for a more easily binding to tumor cells via the multivalent linkages and more readily internalization through the FR-mediated endocytosis. Our study can provide a strategy to quantitatively control the active targeting of nanocarriers to tumor cells for cancer therapy.

1. Introduction

Chemotherapeutic agents are widely used to treat cancer patients but limited due to their systemic toxicity and low efficacy by the nonspecific distribution of the anti-cancer drug [1]. Drug delivery based on nanotechnology has been developed to overcome these limitations due to their enhanced therapeutic efficacy and reduced side effect [2,3]. However, nonspecific cellular targeting and poor cellular internalization of nanocarriers reduce the therapeutic efficacy of the encapsulated agents [4–6]. Hence, novel actively targeting drug delivery systems are urgently needed.

Magnetic-guided and ligand-mediated targeting delivery systems are two representatives of the reported targeting delivery systems and are most frequently concentrated in cancer chemotherapy. As one major targeting approach, magnetic targeting [7–11] is ideally suited to rapidly deliver antibody or fluorescent probes into the targets [12–14]. Furthermore, it is also favorable for distant control, which is greatly easy for the operations in clinical therapy [15]. However, this passive targeting approach does not help the drug systems to specifically interact with the targeted cells, thus resulting in drug expulsion and multiple drug resistance (MDR) [16]. On the other hand, the active targeting [17–23] provides the selective delivery of drugs to targeted cells via the specific ligand recognition to receptors as well as the specific cellular uptake via the receptor-mediated endocytosis [24]. The drug-loaded ligand-targeted nanoparticles are able to effectively overcome MDR [25]. However, the long way for these nanocarriers to reach the tumor sites remarkably lowers the efficiency of the loaded drugs while their off-target toxicities are not simultaneously reduced [26–28]. Considering the advantages and disadvantages of these two targeting strategies, people have combined magnetic nanoparticles (MNPs) and affinity ligands together and developed a dual targeting drug delivery system, which has become imperative since it displays the enormously increased specificity towards cancer cells but the minimum toxicity to the surrounding normal tissues [29–32].

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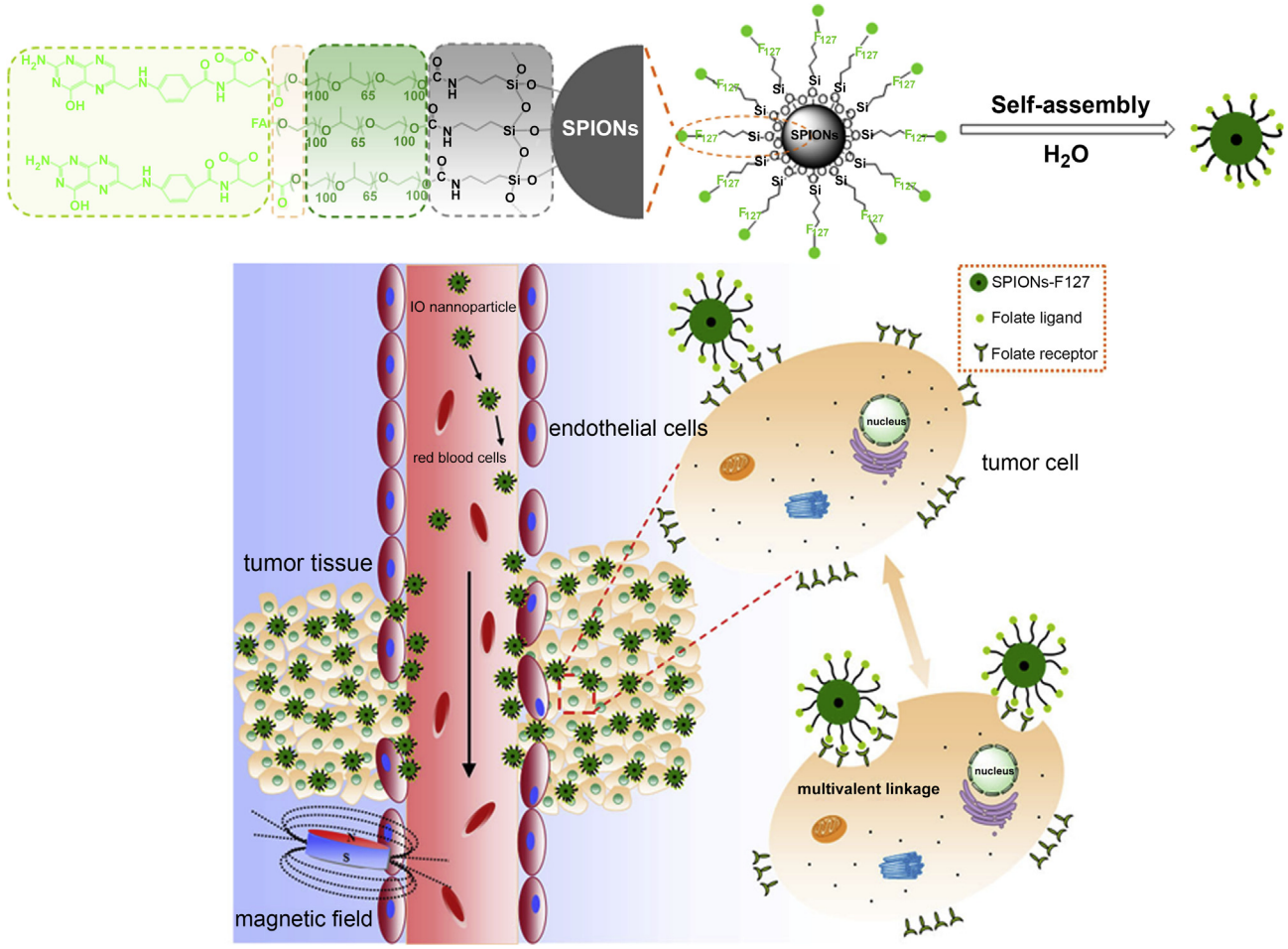
Among the various types of ligands used for the active targeting, folate (FA) stands out to be a desirable choice for malignant tumors [18,29], since most solid tumor cells express a high level of folate receptors (FRs) on their surfaces while the levels of FRs are much lower in non-epithelial tumors and normal tissues [33–35]. Various species of FA-decorated nanoformulations have been developed for the site-specific targeting to carcinomas, such as liposomes [36–38], polymeric micelles [39,40], polymeric nanoparticles [41,42] and dendrimers [43,44].

Interestingly, FR density is known to increase as the stage/grade of the cancer worsens [45]. Quantitative radioligand binding assay has also demonstrated that FR density varies in a wide range in cancer cells. For example, human epithelial mouth carcinoma cell line (KB) and human cervix adenocarcinoma cell line (HeLa) have more than 6 pmol FRs/mg protein [46], approximately equivalent to 280,000 FRs per cell [47]. In contrast, the FR expressers on human lung epithelial cancer cell line (A549) and murine breast cancer cell line (4T1) are less than 2.5 pmol FRs/mg protein (or less than 120,000 FRs per cell) [46]. These information suggest that the density of FA ligands in delivery systems plays an important role in their active targeting delivery, and the definite amount of FA ligands should match with the amount of FRs on the cell membranes. Abnormally high density of FA ligands will promote the non-specific interactions of nanocarriers with cells, increase immunogenicity, and then result in the opsonization-mediated clearance of nanoparticles [16,47]. On the other hand, excessively low FA density cannot generate an effective targeting [47,48]. The main challenge for the targeted delivery is to achieve both the high targeting specificity and the satisfying delivery efficiency but the minimum immune response [16]. Thus, it is very necessary to explore a favorable amount of FA ligands.

In this study, we have developed a dual-targeted drug delivery system by a combination of FA ligands with superparamagnetic iron oxide nanoparticles (SPIONs). SPIONs are firstly modified with the PEO-PPO-PEO (F127) spacer to improve the stability of the nanoparticles in aqueous solution, and subsequently chemically conjugated with different amounts of FA ligands (as shown in Scheme 1). The functionalized SPIONs can self-assemble into magnetic micelles in water. After being injected into blood, these nanocarriers are firstly enriched in the anticipated tumor sites by the magnetic guidance, secondly penetrate into tumor tissues via the well-known enhanced permeability and retention (EPR) effect [49], and finally are bound to FRs on cancer cell membrane through a multivalent linkage and readily internalized into cells via receptor-mediated endocytosis. We have then investigated the active targeting efficiency of this dual targeted nanoparticles to tumor cells both *in vitro* and *in vivo*, especially focusing on the influence on their active targeting ability when the FA ligand density of the MNPs is altered.



Scheme 1. Schematic illustration of the fabrication of the dual-targeted drug delivery system (the FA-targeted MNPs) and the procedure of the cellular targeting: i) the FA-targeted MNPs are accumulated at the tumor site by the EPR effect and the magnetic guidance; ii) the FA-targeted MNPs bind with the folate receptors (FRs) on tumor cell membrane via multivalent linkages, leading to a specific internalization through the FR-mediated endocytosis.