Controlling Ligand Surface Density Optimizes Nanoparticle Binding to ICAM-1

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ABSTRACT: During infection, pathogens utilize surface receptors to gain entry into intracellular compartments. Multiple receptor–ligand interactions that lead to pathogen internalization have been identified and the importance of multivalent ligand binding as a means to facilitate internalization has emerged. The effect of ligand density, however, is less well known. In this study, ligand density was examined using poly(DL-lactic-*co*-glycolic acid) nanoparticles (PLGA NPs). A cyclic peptide, cLABL, was used as a targeting moiety, as it is a known ligand for intercellular cell adhesion molecule-1 (ICAM-1). To modulate the number of reactive sites on the surface of PLGA NPs, modified Pluronic® with carboxyl groups and Pluronic® with hydroxyl groups were combined in different ratios and the particle properties were examined. Utilizing a surfactant mixture directly affected the particle charge and the number of reactive sites for cLABL conjugation. The surface density of cLABL peptide increased as the relative amount of reactive Pluronic® was increased. Studies using carcinomic human alveolar basal epithelial cells (A549) showed that cLABL density might be optimized to improve cellular uptake. These results complement other studies, suggesting that surface density of the targeting moiety on the NP surface should be considered to enhance the effect of ligands used for cell targeting. ©2010 Wiley-Liss, Inc. and the American Pharmacists Association
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Keywords: ligand density; poly(DL-lactic-*co*-glycolic acid) nanoparticles; cLABL; Pluronic®; binding and cellular uptake

INTRODUCTION

The interaction between leukocyte function associated antigen-1 (LFA-1), present on lymphocytes such as T cells, and intercellular cell adhesion molecule-1 (ICAM-1), on antigen presenting cells (APCs), represents an interesting therapeutic target.[1–3] This interaction is a part of T-cell activation through the immunologic synapse, which forms at the interference between T cells and APCs.[3–6] The expression of ICAM-1, which is a ligand for LFA-1, has been investigated in a variety of cells.[1,7] Different cytokines can upregulate ICAM-1 on the surface of cells.[2,7–8] Reports have also suggested a link between tumor growth attenuation and overexpression of ICAM-1 induced by inflammatory cytokines such as tumor necrosis factor-α (TNF-α).[1–2,7–8] ICAM-1 upregulation has been observed in many different inflammatory diseases and cancers cells, such as melanomas, lymphomas, and lung carcinomas, which makes this receptor an interesting target for therapeutic delivery.[1,8]

Peptides derived from binding domains of LFA-1 and ICAM-1 have been developed as ligands for the opposing receptors. Cyclo(1,12)-PenITDGEATDSGC (cLABL), a cyclic peptide, is derived from I-domain of α_L-subunit of LFA-1 integrin. This peptide can inhibit homotypic and heterotypic T-cell adhesion to epithelial and endothelial cells by blocking the LFA-1–ICAM-1 interaction.[9] Furthermore, cLABL has been employed as a targeting ligand for intracellular

Additional Supporting Information may be found in the online version of this article. Supporting Information

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delivery.[2,10] Accordingly, cLABL conjugated to poly(DL-lactic-*co*-glycolic acid) nanoparticles (PLGA NPs) was designed to target anticancer drugs to carcinomic human alveolar basal epithelial cells, A549, via ICAM-1.[1]

Understanding the ligand–receptor interactions between cells or other biological components can be helpful to enhance the efficiency of targeting systems by improving binding avidity and cellular uptake. The structure of viruses such as adenovirous type 2 and the surface density of their ligands have been studied to surmise the importance of multivalent ligands on binding efficiency.[11] Studies showed that penton base proteins presented on adenovirus type 2 cover the surface of these viruses with regular spacing.[11] The surface density presented by this virus may substantially improve binding avidity to the cell receptors. The simulation of the natural structure of viruses based on their ligand density has been utilized to engineer targeted clustered ligands.[11–12] Studies showed that the optimum distribution of ligands on the surface of viruses is a key factor for binding and cellular uptake. Control of ligand density on NPs should provide a means to increase receptor binding avidity and cellular uptake.

The effect of ligand density (e.g., peptide conjugated to NPs) on binding and cellular uptake has not been thoroughly explored. Reports have shown that increasing conjugated ligands on particles often increases the cellular uptake.[13–15] In general, these studies showed that ligands must be present on the surface of NPs above a minimum threshold for binding to occur.[16] However, a few studies also showed that dense surface coverage may not offer expected improvements in binding and cellular uptake.[13–16]

In this study, the effect of ligand density on receptor binding and cellular uptake was investigated using surface-modified PLGA NPs exhibiting conjugated cLABL peptide. To control the number of reactive sites for conjugation on the surface of NPs, mixtures of two surfactants were used during NP fabrication. Such surface modification provided a controllable number of reactive sites on the surface of the NPs for peptide conjugation, allowing us to modulate the peptide surface density on the NPs as a means to optimize binding and cellular uptake. Binding experiment that uses A549 cells was utilized to evaluate the effect of ligand density on NP binding to the cells.

EXPERIMENTAL

Materials

Polymers of poly(DL-lactic-*co*-glycolic acid) (50:50) [PLGA with inherent viscosities of 0.22 dL/g ($M_w \approx 6.7$ kDa; carboxyl terminal group) and 1.05 dL/g ($M_w \approx 101$ kDa)] were purchased from LACTEL Absorbable Polymers International (Pelham, Alaska). Pluronic® F38 ($M_w \approx 4700$ Da), Pluronic® F68 ($M_w \approx 8400$ Da), Pluronic® F108 ($M_w \approx 14{,}600$ Da), and Pluronic® F127 ($M_w \approx 12{,}600$ Da) were obtained from BASF Corporation (Mount Oliver, New Jersey). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimidehydrochloride (EDC) and *N*-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Thermo Scientific (Rockford, Illinois). Coumarin-6 was purchased from Polysciences, Inc. (Warrington, Pennsylvania). Tetrahydrofuran (THF), triethylamine, acetone, diethyl ether, 1×Tris/EDTA buffer solution (pH 8), sodium hydroxide, and ethyl alcohol were obtained from Fisher Scientific (Fair Lawn, New Jersey). D-Mannitol, (dimethylamino)pyridine (DMAP), succinic anhydride, and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, Missouri). cLABL was synthesized in-house by using a reported method.[1–2] Millex™ syringe-driven filter unit (MCE 0.45 μm membrane filter) was purchased from Millipore Corporation (Bedford, Massachusetts). Dialysis membrane (molecular weight cutoff = 100,000) was obtained from Spectrum Laboratory Products Inc. (Rancho Dominguez, California). F-12K medium and A549 cell line were purchased from American Type Culture Collection (Manassas, Virginia). Recombinant, human TNF-α was obtained from Promega (Madison, Wisconsin). BCA™ protein assay kit was obtained from Thermo Scientific (Rockford, Illinois). Costar® 3596 and Costar® 3631 were purchased from Corning Incorporated (Corning, New York).

Conversion of Terminal Hydroxyl Groups to Terminal Carboxyl Groups on Pluronic®

To conjugate cLABL to Pluronic®, the terminal hydroxyl groups were converted to carboxyl groups on Pluronic® F38, F68, F108, and F127.[1–2,17] First, 12.87 mM of each surfactant was dissolved in 10 mL of THF. Then, DMAP (24.5 mg), triethylamine (27 μL), and succinic anhydride (200 mg) were added to the solution. The mixture was kept on a stirrer for 48 h at room temperature in a closed bottle. THF was then removed using a rotary evaporator (Rotoevaporator-R; Büchi, Schweiz, Switzerland) and the product was dissolved in about 15 mL of carbon tetrachloride. Filtration was utilized to remove the excess succinic anhydride (0.45 μL cutoff). Each type of Pluronic® with terminal carboxyl groups was purified by precipitation with 25 mL of diethyl ether and recrystallized with 10 mL of ethyl alcohol. Rotary evaporation was performed to remove the solvents after precipitation and recrystallization. Finally, the products were kept in a vacuum chamber overnight to remove the solvents. Modified Pluronic®s were characterized by ^{1}H NMR spectroscopy (Bruker AVANCE 400 MHz