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Technical note

Impact of surfactants on the target recognition of Fab-conjugated PLGA nanoparticles

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ABSTRACT

Targeted drug delivery with nanoparticles (NPs) requires proper surface ligand presentation and availability. Surfactants are often used as stabilizers in the production of targeted NPs. Here, we evaluated the impact of surfactants on ligand functionalization and downstream molecular recognition. Our model system consisted of fluorescent poly(lactic-co-glycolic acid) (PLGA) NPs that were nanoprecipitated in one of a small panel of commonly-used surfactants followed by equivalent washes and conjugation of an engineered Fab antibody fragment. Size, polydispersity index and zeta potential were determined by dynamic light scattering and laser Doppler anemometry, and Fab presence on the NPs was assessed by enzyme-linked immunosorbent assay. Most importantly, Fab-decorated NP binding to the cell surface receptor was monitored by fluorescence-activated cell sorting. 2% polyvinyl alcohol, 1% sodium cholate, 0.5% Pluronic F127 (F127) and 2% Tween-80 were initially tested. Of the four surfactants tested, PLGA NPs in 0.5% F127 and 2% Tween-80 had the highest cell binding. These two surfactants were then retested in two different concentrations, 0.5% and 2%. The Fab-decorated PLGA NPs in 2% F127 had the highest cell binding. This study highlights the impact of common surfactants and their concentrations on the downstream targeting of ligand-decorated NPs. Similar principles should be applied in the development of future targeted nanosystems where surfactants are employed.

1. Introduction

Targeted drug delivery aims to accumulate a drug at a specific diseased location in the body (e.g. solid tumor) while avoiding healthy host tissue [1]. This can be accomplished by tethering a ligand to a drug or to a nanoparticle (NP) encapsulating a drug. Antibodies, or their fragments, are often employed as the targeting ligand due to their high specificity and affinity to a plethora of (bio)molecules [2]. In general, genetic engineering and recombinant protein technology is often applied to therapeutic antibodies for enhanced pharmacology (e.g. increased affinity or circulation times) [3]. Though, in the specific case of antibody-conjugated NPs, the above technologies can be exploited to mutate and express the antibody to display specific residues for site-directed conjugation strategies that ensure proper orientation of the antigen-binding domain to maximize target recognition [2].

Surfactants, herein, are referred to as the stabilizing agents used during the production of NPs. However, they are often broadly defined as all “surface active agents”. Surfactants act by decreasing the interfacial tension between the components in a material system to increase such properties as miscibility and dispersion [4]. Poly(lactic-co-glycolic acid) (PLGA), a polymer often used in nanomedicine due to its biodegradability, biocompatibility, FDA/EMA approval and malleability to surface modification [5], is one material that frequently employs surfactants during NP production. Although Menon et al. studied the impact of surfactants on the physical and biological properties of PLGA NPs [6], to our knowledge, no studies have investigated the impact of surfactants on the specific targeting of ligand-decorated NPs. Thus, we aimed to produce fluorescent PLGA NPs in different common

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surfactants followed by conjugation of a Fab antibody fragment that targeted a specific cell surface receptor. The NPs were then characterized for their physicochemical qualities, ligand presence and target recognition.

2. Materials and methods

2.1. Materials

The core PLGA (50:50 LA:GA; 44,000 Da; 5004A) was kindly provided by Corbion. PLGA-FKR648 (20,000–30,000 Da) and PLGA (30,000 Da)-polyethylene glycol (5000 Da)-maleimide (PLGA-PEG-Mal) were purchased from Polyscitech/Akina. The polyvinyl alcohol (PVA), sodium cholate (Na Chol) and Tween-80 were purchased from Sigma while the Pluronic F127 (F127; aka Kolliphor Poloxamer 407) was from BASF. The Amicon centrifuge filters (100 kDa MWCO) and the horse radish peroxidase (HRP)-conjugated goat anti-human secondary antibody (anti-human-HRP) were from EMD Millipore. The human anti-human CD44v6 Fab (AbD15179) [7] and a Fab without specificity to CD44v6 was commercially re-engineered to express three C-terminal cysteines by Bio-Rad, termed "v6 Fab" and "(−) Fab", respectively. The medium protein-binding 96-well plates were from Greiner. All cell culture components and TMB substrate were purchased from Life Technologies, and all miscellaneous chemicals for buffer preparation were from Fisher Chemical or Sigma.

2.2. Generation of PLGA nanoparticles by nanoprecipitation

The PLGA NPs were produced by nanoprecipitation (modified from [8]) according to the following percentages: 80% core PLGA, 10% PLGA-FKR648 and 10% PLGA-PEG-Mal. FKR648, a red channel fluophore, was incorporated so that the GFP from the cells would not interfere with detection of the NP fluorescence. The surfactants were prepared in ultrapure water. The percentages of PVA, Na Chol and F127 were based on weight:volume while Tween-80 was based on volume:-volume. Briefly, 20 mg of total PLGA was dissolved in 1 mL acetone for ~1 h and slowly injected (~3–4 mL/min) through a 25 g needle into 20 mL of the respective surfactant at the indicated percentage while stirring at 200 rpm. The mixture was kept stirring for an additional 3–4 hr for solvent evaporation. In the initial study, the PLGA NPs were washed three times by centrifugation at 10,000*g* for 20 min followed by resuspension in 10 mL ultrapure water. In the follow-up study with F127 and Tween-80, the NPs were washed three times with water using Amicon centrifuge filters (100 kDa MWCO) at 3000*g*, being further collected from the filter. All reactions and washes were performed at room temperature. The final concentrations before conjugation (see below) equated to ~2 μg/μL of total PLGA and 2.5 μM maleimide. All PLGA components were stored under argon at −20 °C after usage.

2.3. Fab conjugation to the nanoparticles

Stock v6 and (−) Fabs were diluted until a final concentration of 1 μM in 1X PBS pH 7.4 was obtained. To allow for mild reduction of the C-terminal cysteines, tris(2-carboxyethyl)phosphine (TCEP) was added to the Fabs at a final concentration of 3 μM and incubated for 1–2 hr at 4 °C before addition to the NPs. 50 μg of PLGA was pelleted by centrifugation at 10,000*g* for 10 min. The supernatant was removed, and the pellet was resuspended in the reduced 1 μM Fab solution to maintain a PLGA concentration of ~25 μM (1 μg/μL) and a molar ratio of 1:25 Fab:PLGA (equating to 1 μg of Fab per 20 μg of PLGA). NPs without Fab conjugation were processed similarly but with buffer only. After overnight incubation at 4 °C, the NPs were washed twice by centrifugation at 10,000*g* at 4 °C with 200 μL ultrapure water and finally resuspended in PBS. All NPs were maintained in low light conditions, and each formulation was performed in triplicate. PLGA-FKR648-PEG-Mal NPs were conjugated with the re-engineered, human anti-human CD44v6 Fab are termed "v6 Fab-PLGA NPs", and those conjugated with the human negative control Fab are termed "(−) Fab-PLGA NPs". NPs without any Fab conjugation are termed "bare PLGA NPs".

2.4. Physicochemical characterization of nanoparticles

Dynamic light scattering (DLS) was used to determine size and polydispersity (PdI), and laser Doppler anemometry (LDA) was used to determine the Zeta potential of the NPs. Both DLS and LDA were performed with a Zetasizer Nano ZS (Malvern) using the Smoluchowski model, and three measurements were taken for each of the triplicate NP formulations. Around 3 μg of NPs were diluted in 750 μL of 10 mM NaCl pH 7 for each sample. The data is presented as the average of the triplicate NP formulations and the erros bars represent the standard deviation.

2.5. Monitoring of Fab presence on PLGA NPs by ELISA

Enzyme-linked immunosorbent assay (ELISA) was used to monitor the presence of the Fab on the PLGA NPs. Briefly, 10 μL (~10 μg) of the PLGA NPs themselves were added to 40 μL of 50 mM bicarbonate/carbonate coating buffer pH 9.6 and incubated in a well in a 96-well plate at 37 °C for 3–4 h. After 3X washes with 100 μL PBS containing 0.1% Tween-20 (PBST), 100 μL 1% BSA was added to each well and incubated at 37 °C for 1–2 h. After 3X washes with 100 μL PBST, 50 μL of the anti-human-HRP diluted 1:5000 was added to each well and incubated at 37 °C for 1–2 h. After 3X washes with 100 μL PBST, 50 μL of TMB substrate was added to each well and incubated for ~15 min. The reaction was terminated with 50 μL of 2 M sulfuric acid and immediately read at 450 nm with a microplate reader (Synergy Mx; BioTek).

2.6. Cell culture

The MKN74 cell line was derived from a moderately differentiated tubular adenocarcinoma (intestinal-type carcinoma), and the cells were maintained in RPMI supplemented with 10% (v/v) FBS and 0.5 mg/mL geneticin (to maintain selection pressure; see below) and passaged twice per week diluted 1:10–1:20. The cells were maintained at 37 °C and 5% CO_2 in a water-saturated atmosphere. CD44v6 expression was occasionally confirmed by FACS with anti-CD44v6 monoclonal antibody (MA54; Life Technologies) staining (data not shown) and v6 Fab staining (unpublished data). Mycoplasma detection by PCR was occasionally performed to ensure no contamination, and once during the studies, a short tandem repeat (STR) profile analysis ensured cell line purity. During cell binding studies, the cells were detached with non-enzymatic Versene to maintain the integrity of the surface-bound receptors; however, trypsin/EDTA was used during sub-culture.

2.7. Generation of transfected MKN74 cell lines

The human stomach adenocarcinoma epithelial cell line MKN74 was purchased from the JCRB Cell Bank (Japanese Collection of Research Bioresources Cell Bank). The parental cell line and isogenic variants were kindly provided by C. Oliveira, a co-author of the present work. CD44v3-v10 (variant CD44-04 – ENST00000415148) cloned in a pCMV6-XL5 were purchased from OriGene. These variants were subcloned into a pIRES-EGFP2 expression vector. This vector and the empty version (Mock) were transfected into MKN74 parental cells using Lipofectamine 3000 (Life Technologies) according to manufacturer's instructions. After transfection, CD44v3-v10 and Mock expressing cells were selected with geneticin and then sorted by magnetic bead capture using specific anti-CD44v6 (MA54; Life Technologies) monoclonal