Colloids and Surfaces B: Biointerfaces 171 (2018) 427–436

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Shear stress regulated uptake of liposome-decorated microgels coated with a poly(dopamine) shell

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ARTICLE INFO

Keywords:
Drug release
Cell uptake pathway
Liposomes
Microgels
Multicompartment carriers
Shear stress

ABSTRACT

Advanced multicompartment drug delivery platforms ensure the co-localization of several drugs within the same carrier, thus making it possible to achieve a more effective and safe therapeutic outcome. Herein, we report a novel multicompartment architecture by combining two intrinsically different systems, *i.e.*, polymeric microgels and liposomes, with the aim to achieve different release kinetics for model compounds. We assemble poly(*N*-isopropylacrylamide-*co*-acrylic acid) microgels decorated with liposomes which are subsequently coated with a protective poly(dopamine) shell and a poly(ethylene glycol) (PEG) layer. Since any intravenous administered drug delivery vehicle will get in contact with the dynamics of the blood flow, we evaluate the stealth properties of this novel multicompartment carrier towards protein adsorption and cellular uptake by three relevant cell lines (macrophages, endothelial and cancer cells) under physiological shear stress conditions. Our results demonstrate less protein adsorption for the PEGylated carriers and differences in the extent of internalized carriers depending on the presence of a PEG coating, the studied cell line and the intensity of the applied shear stress. Additionally, we demonstrate that, for all three tested cell lines, shear stress results in the activation of different cell entry pathways as compared to static conditions. All in all, we report a thorough study about the effect of shear stress on the cell association/uptake with a novel multicompartment carrier.

1. Introduction

The construction of multicompartment architectures is an increasingly growing area, since, multicompartment carriers will allow for the encapsulation and subsequent delivery of several (incompatible) therapeutic molecules in a single vehicle [1,2]. Multicompartment carriers guarantee co-localization of different drugs, which can be of utmost importance to achieve the desired therapeutic outcome. If administered separately, attaining a homogeneous spatial and temporal co-delivery at the target site is highly unlikely due to the potential different pharmacokinetics and pharmacodynamics of the different therapeutic compounds.

A number of multicompartment carriers have been reported to date [1,3], being the most thoroughly studied liposomes-in-liposomes [4], polymersomes-in-polymersomes [5] and capsosomes [6], which consist of liposomes entrapped within a polymeric carrier shell. However, all these systems have a common denominator: all the sub-compartments are made of the same nature, which makes the controlled tandem release of cargo challenging. Herein, to combine two inherently different systems with intrinsically different release kinetics, we report a novel multicompartment carrier consisting of a poly(*N*-isopropylacrylamide-*co*-acrylic acid) (P(NIPAM-*co*-AAc)) microgel (MG) core decorated with liposomes and further coated with a protective poly(dopamine) (PDA) shell (Fig. 1). P(NIPAM-*co*-AAc) MG have been the material of choice due to their straight forward preparation, because they can be loaded with both hydrophilic and hydrophobic compounds and they can also be designed to be biodegradable by the right choice of the cross-linker [7].

Since P(NIPAM-*co*-AAc) MG exhibit a fast release profile due to their large pore sizes [8], aiming to achieve a slower release profile, liposomes were chosen as the second type of compartments. Liposomes have attracted considerable attention as drug carriers since they are

Abbreviation: P(NIPAM-*co*-AAc), poly(*N*-isopropylacrylamide-*co*-acrylic acid); MG, microgel; PDA, poly(dopamine); DA, dopamine; PEG, poly(ethylene glycol); PLL-*g*-PEG, poly-*L*-lysine-*graft*-PEG; L, liposomes; MG/L/PDA, Non PEGylated multicompartment carriers; MG/L/PDA/PEG, PEGylated multicompartment carriers; PLL, poly-*L*-lysine; IgG-FITC, FITC-labelled immunoglobulin G; BSA-FITC, FITC-labelled bovine serum albumin; MB, methylene blue; Cal, calcein; MG^{MB}, methylene blue-loaded microgels; L^{Cal}, calcein-loaded liposomes; L^{F}, fluorescently labelled liposomes

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https://doi.org/10.1016/j.colsurfb.2018.07.031
Received 28 May 2018; Received in revised form 12 July 2018; Accepted 16 July 2018
Available online 17 July 2018
0927-7765/

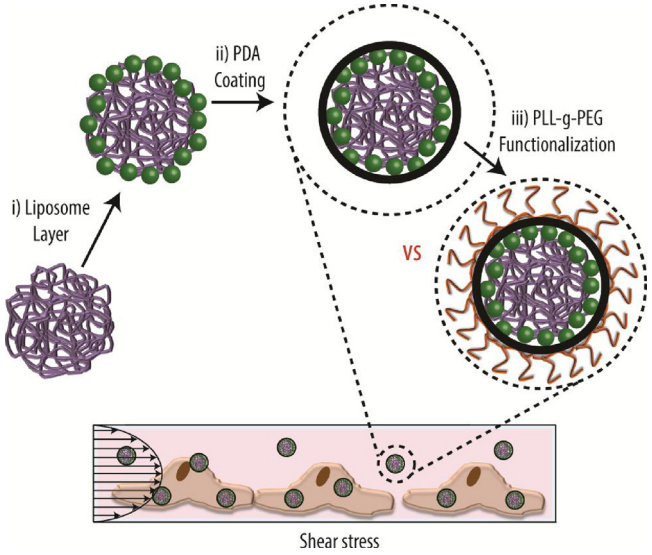

Fig. 1. A microgel core is decorated with a liposome layer (i), followed by the deposition of a polydopamine (PDA) coating (ii). Finally, the surface of the carrier is functionalized with poly-*L*-lysine-*graft*-PEG (PLL-*g*-PEG) (iii). Inset: Evaluation of the PEGylation effect by RAW 264.7 (macrophages), HUVEC (endothelial) and HeLa (cervix cancer) cells under shear stress.

biocompatible, well suited to encapsulate both hydrophilic and hydrophobic compounds and their compositions can be easily tailored to modify surface and charge [9]. However, since liposomes possess low *in vivo* stability, several surface coatings have been reported to enhance their stability [10]. Amongst them, the self-polymerization of dopamine (DA) into PDA has been used as a coating for liposomes rendering them with increased stability [11]. PDA also allows for post-functionalization by means of its ability to react in a straight forward manner with thiols and amines [11].

To maximize drug delivery to tumours, carrier vehicles need to circulate with a long half-life until they reach the target tumour. This effect is usually achieved by decorating the carriers surface with poly (ethylene glycol) (PEG) [12]. PEGylation of our multicompartment carrier is easily achievable by reaction of the PDA coating with the amino groups of the copolymer poly-*L*-lysine-*graft*-PEG (PLL-*g*-PEG) (Fig. 1).

From a different note, upon intravenous injection, drug delivery vehicles are exposed to the dynamics of the circulating blood flow but also of the interstitial fluids and vascular microenvironment in tumours, when targeting cancer is the aim. Both the blood flow and the interstitial fluids in the tumour microenvironment generate mechanical forces such as shear stress. Although it has been previously shown that shear stress can affect the carriers cytotoxicity [13], PEGylation efficiency [11] or the carriers association/interaction with cells [14], including shear stress in *in vitro* set-ups is still rarely seen in literature. This fact could partially explain the poor transition from *in vitro* to preclinical studies [13]. Microfluidic devices are great tools to generate a relevant physiological dynamic environment to assess the performance of the developed carrier [15]. They offer the possibility to closely studying the interactions between drug delivery carriers and biological systems by accurately controlling the fluidic conditions resulting in improved *in vitro* set-ups [16]. Their relevance has been demonstrated by the *in vivo* validation of the findings previously observed using microfluidic devices [17].

Herein, aiming to address these challenges, we i) assemble P(NIPAM-*co*-AAc)/liposomes/PDA carriers; ii) functionalize them with PLL-*g*-PEG to obtain a low-fouling effect; iii) assess the PEGylation efficiency in terms of protein adsorption; iv) evaluate the release profiles of two model compounds encapsulated in independent compartments of the carriers; v) assess the interaction of the multicompartment carriers with three different cell lines (*i.e.*, endothelial cells, macrophages and cancer cells) in the presence of shear stress; and, finally, vi) evaluate the influence of shear stress in the cell internalization pathway of the multicompartment carriers.

2. Methods

2.1. Assembly and characterization of the multicompartment carriers

P(NIPAM-*co*-AAc) MG and liposomes (L) were assembled and characterized as described in the Supplementary Material. For the preparation of non-PEGylated carriers (MG/L/PDA), a suspension of 958 μg MG in Tris 1 (10 mM Tris, pH 8.5) was incubated with L (0.25 mg lipids) for 1.5 h. Next, the suspension was incubated for 16 h in a DA solution (1 mg mL^{-1} in Tris 1) followed by 2× washing cycles in Tris 2 (10 mM Tris and 150 mM NaCl, pH 7.4). For the PEGylated carriers (MG/L/PDA/PEG), the MG/L/PDA assemblies were incubated with PLL-*g*-PEG (1 mg mL^{-1} in Tris 1) for 1 h and washed 2× in Tris 2 to stop the DA self-polymerization.

2.1.1. Differential interference contrast (DIC) and fluorescence microscopy

The samples were imaged with an Olympus Inverted IX83 microscope equipped with a 60× oil-immersion objective.

2.1.2. Size distribution

The diameters of the carriers were determined by measuring at least 200 particles in five independent DIC images using an imaging software (Image J).

2.1.3. Zeta (ζ)-potential

The ζ-potential of all the assemblies was measured in Milli-*Q* water using a ZetaPALS ζ-potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA).

2.1.4. Quartz crystal microbalance with dissipation (QCM-D) monitoring

The deposition of the different layers on a silica crystal (QSX300, Q-sense) was monitored using a Q-sense E1 instrument (Biolin Scientific, Sweden). For details see Supplementary Material. Briefly, first, a poly-*L*-lysine (PLL) solution (1 mg mL^{-1} in Tris 1) was loaded in the flow module reaching surface saturation. After washing the PLL excess, a suspension of 350 nm-sized MG (7.2 mg mL^{-1} in Tris 1) was loaded reaching surface saturation and followed by a washing step. The L were then loaded also reaching surface saturation. Upon washing, a DA solution (1 mg mL^{-1} in Tris 1) was loaded for 1 h. Finally, a PLL-*g*-PEG solution (1 mg mL^{-1} in Tris 1) was also loaded reaching surface saturation. Dissipation and normalized frequency values using the third harmonic are reported.

2.2. Protein adsorption onto multicompartment carriers

A suspension of MG/L/PDA or MG/L/PDA/PEG (0.47 mg in 0.2 mL) was incubated at 37 °C for 4 h in FITC-labelled immunoglobulin G (IgG-FITC) or bovine serum albumin (BSA-FITC) solutions (0.5 mg mL^{-1} in PBS) and washed 3× in PBS. The fluorescence intensity readings of the bound proteins onto the carriers were evaluated by flow cytometry (BD Biosciences, Sparks, MD, USA). For protein adsorption under the presence of shear stress (τ), we employed the same concentration of protein-to-carrier as in static conditions. In particular, syringes containing 17.6 mg of carriers suspended in a 0.5 mg mL^{-1} protein solution in PBS (7.5 mL) were connected to a chamber (μ-slide $VI^{0.4}$, tissue culture treated, Ibidi GmbH. Munich, Germany) applying two different shear stresses ($\tau 0.5 = 0.5$ dyn cm^{-2} and $\tau 4 = 4$ dyn cm^{-2}) with an Ibidi Pump System (Ibidi GmbH, Munich, Germany). At least 20 000 events were analyzed in two independent experiments.