Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Anisotropic biodegradable lipid coated particles for spatially dynamic protein presentation

Randall A. Meyer [a,b,c,1], Mohit P. Mathew [a,b,1], Elana Ben-Akiva [a,b], Joel C. Sunshine [a,b], Ron B Shmueli [a,b], Qiuyin Ren [a,b], Kevin J. Yarema [a,b], Jordan J. Green [a,b,c,d,e,f,g,h,*]

[a] Department of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA
[b] Translational Tissue Engineering Center, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA
[c] Institute for Nanobiotechnology, Johns Hopkins University, Baltimore, MD 21231, USA
[d] Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD 21231, USA
[e] Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA
[f] Department of Oncology, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA
[g] Department of Neurosurgery, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA
[h] Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21231, USA

ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form 29 March 2018
Accepted 30 March 2018
Available online xxxx

Keywords:
Lipids
Polymers
Membrane fluidity
Particle shape
Biomimetic

ABSTRACT

There has been growing interest in the use of particles coated with lipids for applications ranging from drug delivery, gene delivery, and diagnostic imaging to immunoengineering. To date, almost all particles with lipid coatings have been spherical despite emerging evidence that non-spherical shapes can provide important advantages including reduced non-specific elimination and increased target-specific binding. We combine control of core particle geometry with control of particle surface functionality by developing anisotropic, biodegradable ellipsoidal particles with lipid coatings. We demonstrate that these lipid coated ellipsoidal particles maintain advantageous properties of lipid polymer hybrid particles, such as the ability for modular protein conjugation to the particle surface using versatile bioorthogonal ligation reactions. In addition, they exhibit biomimetic membrane fluidity and demonstrate lateral diffusive properties characteristic of natural membrane proteins. These ellipsoidal particles simultaneously provide benefits of non-spherical particles in terms of stability and resistance to non-specific phagocytosis by macrophages as well as enhanced targeted binding. These biomaterials provide a novel and flexible platform for numerous biomedical applications.

Statement of significance

The research reported here documents the ability of non-spherical polymeric particles to be coated with lipids to form anisotropic biomimetic particles. In addition, we demonstrate that these lipid-coated biodegradable polymeric particles can be conjugated to a wide variety of biological molecules in a "click-like" fashion. This is of interest due to the multiple types of cellular mimicry enabled by this biomaterial based technology. These features include mimicry of the highly anisotropic shape exhibited by cells, surface presentation of membrane bound protein mimetics, and lateral diffusivity of membrane bound substrates comparable to that of a plasma membrane. This platform is demonstrated to facilitate targeted cell binding while being resistant to non-specific cellular uptake. Such a platform could allow for investigations into how physical parameters of a particle and its surface affect the interface between biomaterials and cells, as well as provide biomimetic technology platforms for drug delivery and cellular engineering.

1. Introduction

Lipid polymer hybrid particles, that combine the biomimetic cellular surface features of a liposome with the structural support

* Corresponding author at: Department of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA.
E-mail address: green@jhu.edu (J.J. Green).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.actbio.2018.03.056
1742-7061/

and stability of a polymeric particle, have been of great interest to the biomaterials community in recent years. Generally, these constructs are of core-shell design with the polymer encapsulating various therapeutics in the core, and naturally or synthetically derived lipids forming a shell. By fusing a preformed lipid vesicle to a polymeric particle [1] or taking advantage of self-assembling lipid bilayers during particle synthesis [2], these particles can be fabricated with a variety of different strategies depending on the desired application.

The lipid polymer hybrid particle technology holds tremendous promise and has already been demonstrated in several applications [3] including drug delivery [4], diagnostic imaging [5], and gene delivery [6,7]. Furthermore, this lipid coating strategy has been extended to the synthesis of polymeric nanoparticles containing membranes derived from red blood cells [8], platelets [9], and cancer cells [10]. Polymeric particles with naturally derived membranes have been shown to be useful for many other applications including adsorption of hemolytic toxins [11], pathogen binding [9], and cancer cell antigen delivery for vaccines [10].

One particle design limitation of this approach, however, has been that, up to now, almost all lipid coated particles have been of isotropic, spherical shape. Yet, emerging evidence suggests that that non-spherical micro- and nanoparticles possess several key advantages over traditional spherical particles which include inhibited non-specific cellular uptake [12] and simultaneously potential enhanced target-specific binding and cell uptake [13]. As a result, anisotropic particles have been appropriated for several recent biological applications such as anti-cancer drug delivery [14,15], gene delivery *in vitro* and *in vivo* [6,16,17], and immunoengineering to stimulate T-Cells against tumor associated antigens [18,19]. In all of these applications, the non-spherical anisotropic particle has been shown to be superior to the isotropic spherical particle.

To investigate the feasibility of combining these previously separate particle technologies—the use of anisotropic shapes in particle core design and the hybridization of lipids on polymeric particles for dynamic surfaces—we developed a procedure to reproducibly generate non-spherical, ellipsoidal lipid coated particles with a biodegradable polymer support. The process includes generating non-spherical particles, which can be manufactured from both top-down [13] and bottom-up methods [20]. In the current work, we utilized, as outlined in Scheme 1, a thin film stretching method developed by Ho et al. [21] that we recently automated [22] with an electromechanical stretching device to robustly generate ellipsoidal anisotropic microparticles to serve as the support for the lipids. Next, the ellipsoidal lipid coated particles were generated by fusing 200 nm liposomes to these particles under sonication. Subsequently, the lipid surfaces were functionalized in a flexible manner through the use of biotinylated biomolecules. These new biomaterials, anisotropic biodegradable particles that exhibit resistance to non-specific cellular internalization and enable spatially dynamic protein presentation from their surfaces, are promising as biotechnology devices for delivery and diagnostic applications (see Scheme 2).

2. Materials and methods

2.1. Particle preparation and characterization

Acid terminated poly(lactic-co-glycolic acid) (PLGA – 85:15 L:G ratio, MW 45,000 Da – 55,000 Da) (Akina Inc.; West Lafayette, IN) was dissolved in 5 mL of dichloromethane (DCM) at a concentration of 20 mg/mL. In order to visualize particles under fluorescence microscopy, 7-amino-4-methyl coumarin (7-AMC- Sigma-Aldrich; St. Louis, MO) or Nile Red (Life Technologies; Grand Island, NY) were added to the DCM solution at a 1% w/w ratio to the polymer. The resulting solution was homogenized by a T-25 digital ULTRA-TURRAX IKA tissue homogenizer at 5,000 rpm for 1 min in 50 mL of 1% poly(vinyl alcohol) (PVA) solution (IKA Works; Wilmington, NC). The subsequent emulsion was then transferred to 100 mL of 0.5% PVA solution agitated by magnetic stirbar and the DCM was allowed to evaporate over the course of 4 h. The suspended particles were centrifuged out of solution at 3000*g* for 5 min and washed 3 times with water. The resulting particles were flash frozen in liquid nitrogen and lyophilized prior to use.

To synthesize non-spherical ellipsoidal particles, we utilized the thin film stretching method developed by Ho et al [21]. Spherical particles synthesized by emulsion were suspended into a solution of 10% PVA and 2% glycerol at a concentration of 5 mg/mL and 10 mL of this solution was deposited into a rectangular petri dish. The film was allowed to dry overnight, and the next day the film was cut to size and mounted onto an automated thin film stretching device [22]. The entire apparatus was heated up to 90 °C and the film was stretched 2-fold in one direction to produce ellipsoidal particles with a major axis roughly 2 times the original particle diameter and a minor axis roughly 0.7 times the original particle diameter. After stretching, the film was allowed to cool back down to room temperature and then was dissolved in water. Particles were washed and subsequently lyophilized prior to use and characterization.

Particle characterization was conducted using scanning electron microscopy (Leo FESEM). Lyophilized particles were mounted onto an aluminum tack (Electron Microscopy Services; Hatfield, PA) using carbon tape (Nisshin EM Co.; Tokyo, Japan). The particles were then sputter coated with 30 nm of gold-palladium alloy. After sputter coating, the particles were imaged by SEM. Particle size and aspect ratio data were obtained by ImageJ analysis of the subsequent SEM images.

2.2. Lipid coated particle preparation and imaging

Non-spherical lipid coated particles were prepared utilizing a two-step method similar in concept to what has previously described for spherical particles [23]. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and cholesterol (Avanti Polar Lipids; Alabaster, AL) were mixed into a 70:30 w/w ratio. For fluorescent lipid imaging studies, rhodamine conjugated DOPC (Avanti Polar Lipids; Alabaster, AL) was mixed with DOPC, and cholesterol in a 1:69:30 w/w ratio. For surface functionalization, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide] (MCC-DOPC) (Avanti Polar Lipids; Alabaster, AL), DOPC, and cholesterol were mixed in a 35:35:30 w/w ratio. A total of 1 mg of the lipids was aliquoted and left to dry into a thin film overnight under a vacuum. The lipids were then hydrated in 1 mL of water. The lipids were heated to 60 °C and extruded through a 200 nm filter (Avanti Polar Lipids; Alabaster, AL). Liposome formation was verified with sizing at 200 nm using dynamic light scattering (Malvern Instruments; Westborough, MA). The liposomes were then mixed with spherical or non-spherical particles (in a 33.4 μg liposome to 1 mg particle ratio) and sonicated for 30 s at 2 W power in a 1.5 mL Eppendorf tube. Temperature was maintained at 4 °C with an aluminum cooling block (Light Labs; Dallas, TX). The subsequent lipid coated particles were purified from solution through centrifugation at 4 °C for 5 min at 300*g*. After three washes, the lipid coated particles were stored at 4 °C until further use.

To analyze the formation of lipid constructs on the particle surface, confocal imaging of PLGA particles encapsulating 7-AMC were coated with rhodamine lipid containing liposomes. Confocal image acquisition was completed with a Zeiss 780 FCS Confocal Microscope. To derive profile information, we used the ImageJ profile

Please cite this article in press as: R.A. Meyer et al., Anisotropic biodegradable lipid coated particles for spatially dynamic protein presentation, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.03.056