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Monocyte-mediated delivery of polymeric backpacks to inflamed tissues: a generalized strategy to deliver drugs to treat inflammation

Aaron C. Anselmo [a,1], Jonathan B. Gilbert [b,1], Sunny Kumar [a], Vivek Gupta [c], Robert E. Cohen [b], Michael F. Rubner [d], Samir Mitragotri [a,*]

[a] Department of Chemical Engineering, Center for Bioengineering, University of California, Santa Barbara, CA 93106, United States
[b] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States
[c] School of Pharmacy, Keck Graduate Institute, 535 Watson Dr., Claremont, CA 91711, United States
[d] Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States

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ABSTRACT

Targeted delivery of drugs and imaging agents to inflamed tissues, as in the cases of cancer, Alzheimer's disease, Parkinson's disease, and arthritis, represents one of the major challenges in drug delivery. Monocytes possess a unique ability to target and penetrate into sites of inflammation. Here, we describe a broad approach to take advantage of the natural ability of monocytes to target and deliver flat polymeric particles ("Cellular Backpacks") to inflamed tissues. Cellular backpacks attach strongly to the surface of monocytes but do not undergo phagocytosis due to backpack's size, disk-like shape and flexibility. Following attachment of backpacks, monocytes retain important cellular functions including transmigration through an endothelial monolayer and differentiation into macrophages. In two separate *in vivo* inflammation models, backpack-laden monocytes exhibit increased targeting to inflamed tissues. Cellular backpacks, and their abilities to attach to monocytes without impairing monocyte functions and 'hitchhike' to a variety of inflamed tissues, offer a new platform for both cell-mediated therapies and broad targeting of inflamed tissues.

1. Introduction

The demand for the targeted delivery of therapeutics to specific locations in the body has driven tremendous advances in particle-based delivery strategies. These advances include the use of particles with various surface chemistries, targeting ligands, shapes and sizes to direct drug carriers to the desired location in the body [1–3]. Despite these advances, polymeric drug carriers suffer from poor circulation times, rapid clearance by the immune system, inefficient targeting to diseased tissues and limited abilities to cross biological barriers [4,5]. Of these limitations, limited ability to cross biological barriers is particularly challenging since these barriers prevent deep penetration of nanoparticles into tissues. In the case of cancer, this leads to only peripheral accumulation of nanoparticles which limits their therapeutic efficacy since loaded drugs cannot reach tumor cores. This limitation can be addressed with the aid of the body's own circulating white blood cells which are known to cross tumor barriers. Monocytes, a type of white blood cell, target inflamed endothelium and enter inflamed tissues to terminally differentiate into macrophages that reside in a variety of diseased tissues including cancer [6], atherosclerosis [7], and arthritis [8], to name a few. Monocyte-differentiated macrophages potentially make up 50% of the cell tumor mass, even reaching deep hypoxic areas of the tumor [6]. This unique ability of monocytes to cross barriers before penetrating into deep sections of tissues illustrates their unique potential to deliver drugs into otherwise inaccessible areas for numerous pathologies.

Recent studies have demonstrated the ability of monocytes and macrophages to target and deliver therapeutics to inflamed tissues [9]. Macrophages have been used to deliver various cargoes aimed at reducing HIV replication in the brain [10], preventing tumor growth [11], targeting brain metastases [12], and reducing brain inflammation in a Parkinson's disease model by providing neuroprotective capabilities [13]. While related, monocytes are distinct from macrophages, and only recently have monocytes been investigated as vehicles to deliver therapeutic agents to pathological tissues [14]. Unlike macrophages, which are terminally differentiated cells which permanently reside in various tissues, monocytes are circulatory cells and can thus navigate the vasculature and target elusive pathological tissues. However, like macrophages, monocytes are phagocytic cells capable of internalizing foreign materials, including drug loaded polymeric particles. Avoiding this internalization for cell-mediated therapies is of paramount importance since internalization can potentially lead to endosomal degradation of polymeric particles [15] and subsequent loss or alteration of therapeutic efficacy. Recently, the size and shape of polymeric particles has been shown to greatly affect phagocytosis, specifically, non-

* Corresponding author.
E-mail address: samir@engineering.ucsb.edu (S. Mitragotri).
[1] Authors contributed equally.

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spherical particles have been shown to be internalized to a lesser extent than their spherical counterparts [16–18]. By engineering particle shape using these recently discovered physical parameters as a guideline, we have developed particles capable of avoiding phagocytosis by macrophages [19], the cells most capable of phagocytosis, and a variety of other cell types while still remaining bound to the cell's surface [16,20,21]. These flat disk-like, anisotropic polymeric particles, termed 'Cellular Backpacks' (BPs), are hundreds of nanometers thick and microns wide [22,23]. Here we report, based on *in vitro* and *in vivo* experiments in mouse models, the ability of monocyte-hitchhiked BPs to target and penetrate tissues. The BPs, reported here, attach to the surface of monocytes and avoid phagocytosis. Following attachment to monocytes, essential cell functions of monocytes such as differentiability and extravasation through an endothelial cell layer are preserved. *In vivo*, BP-functionalized monocytes target and deliver BPs to two distinct inflamed organs in two separate inflammation models.

2. Materials and methods

2.1. Materials

PMAA (Aldrich, M = 100 kDa) PVPON (Sigma, M = 1300 kDa), PAH (Polysciences, M = 150 kDa), EMG 705 anionic MNP (Ferrotec), PAA (Polysciences, M = 450 kDa) N-hydroxysuccinimide-biotin (Sigma) was used as received. To fabricate PAH-biotin, 25 mg of PAH was dissolved in 50 mM MES buffer at pH 8. 15.3 mg of NHS-biotin was dissolved in DMSO at 22 mg/ml before adding to the PAH solution resulting in ~15% amine substitution. The solution reacted for 4 h before it was purified by dialysis overnight into water.

2.2. Backpack fabrication

The nomenclature for LbL follows $(poly1X/poly2Y)_z$, where X and Y are the pH of the polymer solutions and z is the number of bilayers deposited (1 bilayer = poly1 + poly2). A non-integer value of z indicates the assembly was terminated with poly1. Backpacks (BPs) are formed through a combination of photolithography and polyelectrolyte multilayers as previously described [22]. All BPs tested had the structure of $(PMAA2/PVPON2)_{30.5}$ $(PAH3/MNP4)_{10.5}$ $(PAA4/PAH\text{-}biotin4)_8$. The attachment region (PAA4/PAH-biotin4) had 150 mM NaCl added to the polymer solutions.

2.3. Backpack preparation

Fluorescent (TRITC, FITC) streptavidin (Pierce Thermo) was diluted with 0.1 M (pH 5) sodium acetate to 100 μg/ml. 600 μl streptavidin solution was pipetted and spread uniformly onto glass slides containing BPs for 30 min at room temperature. Glass slides were then washed in 0.1 M sodium acetate (pH 5) for 30s to 1 min and allowed to air dry. Mouse IgG functionalized with biotin, IgG-B (Santa Cruz Biotechnologies), was diluted to 100 μg/ml with PBS and pipetted onto glass slide containing BPs as above. For 3H studies, 3H-labeled biotin (American Radiolabeled Chemicals) was mixed with IgG-B to radiolabel BPs. After 30 min, BPs (in pH 7.4 PBS) were gently scraped off of the glass slide surface and centrifuged at 5000 *g* for 10 min. IgG-B containing supernatant was removed and BPs were washed once in PBS.

2.4. Cell culture

WEHI-265.1 monocytes (ATCC) were incubated at 37 °C in 5% CO_2 in high glucose DMEM, 10% FBS, 1% penicillin/streptomycin, and 0.05 M 2-mercaptoethanol. HUVECs (Invitrogen) were incubated at 37 °C in 5% CO_2 in Medium 200 (M200) media with low serum growth supplement (LSGS) and 1% penicillin/streptomycin. J774 macrophages were incubated at 37 °C in 5% CO_2 in high glucose DMEM, 10% FBS, 1% penicillin/streptomycin. WEHI-3 monocytes were incubated at 37 °C in 5% CO_2 in IMDM, 10% FBS, 1% penicillin/streptomycin, and 0.05 M 2-mercaptoethanol.

2.5. Attachment of BPs to monocytes

A known number of BPs, in suspension, were incubated with monocytes at 37 °C for 30 min in PBS. Unbound BPs were separated from monocyte hitchhiked BPs *via* centrifugation at 200 g. Monocyte hitchhiked BPs were imaged on an Olympus Fluoview 500 (Olympus America Inc.).

2.6. Fluorescence-activated cell sorting (FACS)

BPs, monocytes, and conjugates were suspended in PBS. A FACSAria Flow Cytomer with a 70 μm nozzle was used to quantify attachment of BPs to monocytes. Mammalian cells were filtered prior to analyzing sample.

2.7. MTT assay

WEHI-265.1 monocytes were seeded in a 96-well plate at 1.5×10^4 cells per well for 24 h. BPs, 3 μm polystyrene (PS) particles (Polysciences), and 3 μm IgG-B coated PS particles were incubated with monocytes at a 1:1 (Cell:Particle) ratio. IgG-B was attached to 3 μm PS particles *via* incubation with 100 μg/ml of IgG-B in PBS. 10 μl of MTT reagent was added to each well and allowed to incubate in standard cell culture conditions for 2 h. 100 μl of Detergent Reagent was then added and the plate was incubated in the dark at room temperature for 2 h.

2.8. Differentiation of monocytes

6×10^5 WEHI-265.1 monocytes without BPs attached were incubated in 6 well plates with varying concentrations of phorbol 12-myristate 13-acetate (Sigma), PMA, in standard cell culture media. Analysis for adherence and average diameter of cells was performed using ImageJ. Diameter of cells was determined by taking the longest dimension of a cell. At least 100 cells were used for quantification of cell diameter and at least 10 images were used for analysis of cell adherence. In parallel, WEHI-265.1 monocytes were incubated with FITC labeled BPs and sorted for using a FACSAria Flow Cytomer with a 70 μm nozzle. FACS-sorted monocyte-hitchhiked BPs were incubated in 6 well plates at identical conditions.

2.9. Transmigration assay

HUVECs were plated in the upper chamber of Corning Transwell plates (3 μm, 6 well) at 6×10^5 cells per well in M200 media and allowed to attach and grow to form a complete monolayer (confirmed *via* TEER measurements), after which media was changed to DMEM in all chambers. 6×10^5 WEHI-265.1 monocytes (also in DMEM), either with none or 3×10^5 (1 BP per 2 cells) FITC-labeled BPs, were pipetted on top of the HUVEC monolayer in the top chambers, which was then suspended above the collection chamber containing DMEM with 10 nM Monocyte Chemoattractant Protein-1 (Fisher). Monocytes which passed through the HUVEC monolayer were counted manually on a hemocytometer. BP migration was quantified *via* fluorescent reading (normalized to applied dose) of BPs that had migrated into the lower chamber.

2.10. Inflammation models and biodistribution

Healthy female BALB/c mice (18–20 g) were administered 10 μg of lipopolysaccharide (LPS) in 60 μl saline intranasally or 5 μg of LPS in 30 μl intradermally. ICAM expression in lung inflammation model was measured *via* ELISA (Ray Biotech). 3×10^6 WEHI-3 monocytes