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Macrophages with cellular backpacks for targeted drug delivery to the brain

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ABSTRACT

Most potent therapeutics are unable to cross the blood-brain barrier following systemic administration, which necessitates the development of unconventional, clinically applicable drug delivery systems. With the given challenges, biologically active vehicles are crucial to accomplishing this task. We now report a new method for drug delivery that utilizes living cells as vehicles for drug carriage across the blood brain barrier. Cellular backpacks, 7–10 μm diameter polymer patches of a few hundred nanometers in thickness, are a potentially interesting approach, because they can act as drug depots that travel with the cell-carrier, without being phagocytized. Backpacks loaded with a potent antioxidant, catalase, were attached to autologous macrophages and systemically administered into mice with brain inflammation. Using inflammatory response cells enabled targeted drug transport to the inflamed brain. Furthermore, catalase-loaded backpacks demonstrated potent therapeutic effects deactivating free radicals released by activated microglia *in vitro*. This approach for drug carriage and release can accelerate the development of new drug formulations for all the neurodegenerative disorders.

1. Introduction

The delivery of therapeutics across the blood brain barrier (BBB) has been extremely challenging and is currently a major impediment for the treatment of diseases in the brain. Recently, living cells have been investigated as carriers for actively targeted drug delivery, which has opened new therapeutic avenues within the central nervous system (CNS) [1]. For this strategy to be successful, the efficient penetration across the BBB by cell-carriers is crucial for the success of these cell-based formulations. Some cell types are naturally capable of crossing the BBB, such as leukocytes (neutrophils and monocytes), which can traverse the endothelial wall due to increased margination and extravasation [2] when the BBB breaks down during inflammation [3–6]. These cells appear to traffic primarily between adjacent endothelial cells through the transient opening of tight junctions [7,8], and utilizing diapedesis and chemotaxis [9]. Because of their natural recruitment across the BBB during inflammation, immune cells are an ideal candidate as cellular vehicles for drug delivery.

For maximal efficacy, immune cells should carry therapeutically significant quantities of drug. Traditional strategies often result in poor drug loadings because they are sequestered within the phagosome of macrophages, which potentially degrades the drug and reduces the release rates. Furthermore, accumulated drugs, especially antineoplastic agents, may degrade the survival or migration of the cell-carrier [10], which has created a bottleneck for cell-based therapy. In addition, the small size of payload materials traversing the BBB with cell carriers can limit the amount delivered, with 100–200 nm particles currently being the largest [11]. To circumvent these limitations, cellular backpacks, micron-scale patches of a few hundred nanometers in thickness, were

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fabricated by layer-by-layer (LbL) assembly to be attached to the surface of cell-carriers [12–14]. Due to their shape, size and composition, cellular backpacks are not engulfed by macrophages, making them an attractive strategy that sequesters the cargo outside the cells to protect both the cell and the drug from degradation. Recent work has demonstrated that backpack-monocyte conjugates migrate and accumulate in inflamed tissue sites (e.g. lungs and skin) [15]. These cellular backpacks can be designed to carry toxic loads of a potent anticancer drug without affecting cell's natural functions [16]. Furthermore, cell backpacks loaded with catalase have shown antioxidant properties *in vitro* [17].

In this work, we introduce a new method that allows for non-invasive, safe and efficient access to most secluded sites in the brain. This strategy can be used for treatment of various neurodegenerative disorders, infectious diseases, and other types of brain and cellular injuries. We demonstrate that backpacks loaded with catalase can be attached to macrophages and transported across the BBB in a mouse model of lipopolysaccharide (LPS)-induced encephalitis. This indicates that engaging natural immune cells such as monocyte-macrophages as drug carriers is a potentially new strategy for treating a range of neuroinflammatory and neurodegenerative diseases.

2. Results

2.1. Cell backpacks

Backpacks were constructed by a combination of photolithography and layer-by-layer (LbL) assembly [14], which resulted in disc-shaped polymer patches 7 μm in diameter. As shown in Fig. 1, the cell backpacks were initially fabricated by deposition of various material regions, serially, onto a glass microscope slide. At the interface between the glass slide and the assembled multilayer construct is a “release region” which can dissolve upon incubation with the sugar, melibiose. We had previously developed this as a simple and benign means to releasing backpacks from their substrates [18]. Atop this sacrificial film, we deposited a “magnetic region” to aid in purification of cell backpacks, confer film rigidity and potentially serve as an imaging agent [19–22]. Then we deposited a “payload region” that, in this case, contains catalase, a potent anti-oxidant (More details about catalase payload region can be found in Supplemental Fig. 1). Finally, the top layer is composed of a “cell attachment region”, which contains antibodies that enable the backpacks to specifically attach to desired cell-types. In this instance, we attached macrophage-targeting polyclonal antibodies through avidin-biotin links. Catalase-loaded backpacks could withstand the fabrication processing (See more details on Supplemental Fig. 2).

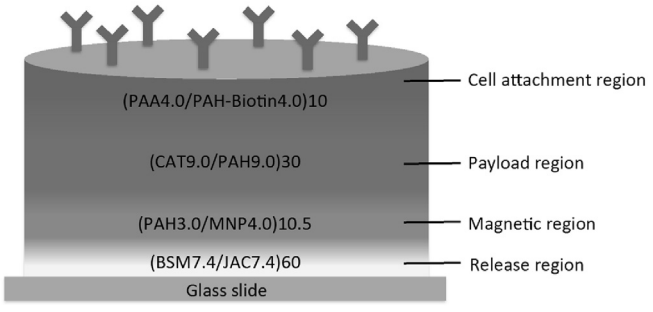

Fig. 1. Scheme showing the catalase containing backpack structure. From bottom to top: Release region is composed of 60 bilayers of bovine submaxillary mucin (BSM) and lectin jacalin (JAC); Magnetic region made of 10.5 bilayers of poly(allylamine hydrochloride) (PAH) and magnetic nanoparticles (MNP); Payload region containing 30 bilayers of PAH and catalase from bovine liver (CAT); Cell attachment region comprised of 10 bilayers of poly(acrylic aid) (PAA) and biotinylated PAH (PAH-biotin). Backpacks are topped with CD11b antibodies for macrophage conjugation.

2.2. Ability for backpacks to cross the BBB in vitro

Cell backpacks were attached to macrophages as follows. Fluorescently-labeled DiO-macrophages (green, 2×10^6 cells/ml) were pipetted onto the surfaces of glass slides with fabricated cell backpacks, which were functionalized with NeutrAvidin Dylight 550 (red). After a brief incubation, macrophages adhered to the backpacks via CD11b antibody attachment, and the glass slides were thrice washed with media to remove non-adhered cells. (See online methods for more details; Supplemental Fig. 3**)**. To detach the macrophage-backpack conjugates, the glass slides were incubated with 1 mL of 100 mM melibiose in PBS for 1 h at 37 °C[18]. The remaining weakly adhered macrophage-backpack conjugates were removed with a cell scraper and re-suspended into saline-buffered solution.

Fig. 2 shows microscope images of macrophages with attached backpacks. For this purpose, macrophages were labeled with a fluorescent dye DiO (green) and pipetted onto the surface of the glass slide with backpacks stained with Neutravidin Dylight 550 (red). Non-adhered cells were removed by rinsing with PBS, and backpack attached macrophages were detached from the glass slide using melibiose. The released cells with backpacks were seeded onto a slide and examined by confocal microscopy. The functionalization protocol yielded 80% conjugation. Noteworthy, backpacks did not affect macrophage viability (Supplemental Fig. 4).

A preliminary *in vitro* test was carried out to show whether cell backpacks were able to cross a cell monolayer. Backpacks alone and those attached to macrophages were incubated in a transwell plate containing a cell monolayer of mouse brain endothelial cells (bEnd.3), and chemoattractant, macrophage chemotactic factor-1 (MCP-1) (150 ng/ml, R&D Systems) that was placed into the lower chamber [19]. Following incubation at 37 °C in a shaker, the bEnd.3 inserts were removed, placed in 24-well plates with fresh media, and centrifuged to pellet the migrating cells to the plate's bottom chamber (See online methods for details). As shown in Fig. 3, some backpacks were able to cross the cell monolayer *in vitro*, but significantly more could do so when attached to macrophages (Fig. 3A), meaning that the macrophages can work by facilitating the passage of backpacks through the BBB. The macrophages themselves are able to cross the cell monolayer *in vitro* and the added bulk from the cell backpacks slightly slows their transfer (Fig. 3B).

2.3. Recruitment of macrophages with the attached backpacks to the brain in LPS-intoxicated mice

To determine whether the macrophages were capable of carrying the cell backpacks across the blood-brain barrier *in vivo*, we used C57/BL mice with LPS-induced brain inflammation as described earlier [23]. Twenty-four hours later, freshly prepared macrophage-backpack conjugates were administered intravenously (5×10^6 cells/mouse in 100 μl). Fourteen hours later, mice were sacrificed, perfused, brains were sectioned, and examined by confocal microscopy. LPS-intoxicated mice injected with backpacks alone (no macrophages) were used as a control group. As shown in Fig. 4A, many macrophage aggregates (green) can be seen all over the brain. Noteworthy, only macrophage aggregates can be observed in that image (single macrophages or single backpacks cannot be observed). To better visualize whether backpacks were present in the brain, zoomed in images (40x) were acquired. Fig. 4**C-E** shows co-localization of fluorescently-labeled