Attachment of Liposomes on Magnetotactic Bacteria Acting as Self-Propelled Drug Delivery Agents

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INTRODUCTION AND OBJECTIVE

Over the past decade significant improvements have been achieved in cancer treatments, resulting in reduced rates of mortality. However, developing new drugs for targeted delivery and deep tumor penetration to reach cancerous cells are limited by significant acute and systemic toxicities (Torchilin 2010). The aim of this study was to develop a potent drug delivery system using MC-1 magnetotactic bacteria (MTB) as a targeted drug delivery system, which can penetrate the hypoxic region of solid tumors. MTB bacteria provide several unique advantages. Firstly, the ideal cell diameter of approximately 2µm enables the bacteria to navigate within the smallest blood vessels. In addition, two bundles of flagella provide 4pN of thrust force for propulsion, allowing a swimming velocity around 200-300 µm/s (Martel 2009). Furthermore, the embedded chain of membrane-based single magnetic domain iron-oxide nano-crystals, called magnetosome, provides directional control while applying a weak external magnetic field (Atsushi 2008). To develop the current drug delivery system, MTB bacteria were covalently functionalized to liposomes containing a therapeutic agent. The nature and structure of the liposome carrier (Fenske 2005) makes them a superior candidate for an efficient interaction with bacterial cell membranes.

MATERIALS AND METHODS

Distearoylphosphatidylcholine (DSPC) and Distearoylphosphatidylethanolamine derivatives of PEG with a terminal carboxyl group (DSPE-PEG-COOH) were purchased from Avanti Polar Lipids (Alabaster, AL). 2-(N-morpholino) ethanesulfonic acid (MES), 1-Ethyl-3-(3-dimethylaminopropyl) -carbodiimide (EDC), N hydroxysulfosuccinimide (sulfo-NHS), Cholesterol (CH) and Sepharose CL-4B were obtained from Sigma-Aldrich (St. Louis, Mo). N-(6Tetramethylrhodaminethiocarbamoyl)-1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine, Triethylammonium Salt (TRITC DHPE) were purchased from Invitrogen (Burlington, CA).

Preparation of liposome

Lipids composed of DSPC/CH (3:2, m/m) with 6mol% of DSPE-PEG2000-COOH were dissolved with 2 mL of a 4:1 (v/v) chloroform–methanol mixture. For fluorescence microscopy studies 2 mol% of TRITC–DHPE were included in the liposome formulation. Multilamellar liposome vesicles (MLVs) were first prepared by a reverse-phase evaporation technique. The film was hydrated with a 100mM MES buffer (pH5.5). To obtain small unilamellar vesicles (SUVs), the MLV suspension was repetitively passed through a 200 nm polycarbonate membrane. Uniform vesicles were obtained with an average size of 170.23 ± 2.8 nm and a polydispersity index of 0.03.

Attachment of liposome to the MTB

Carboxylated liposomes were conjugated to MTB by EDC chemistry (Sun 2011).The attachment was based on the availability of free amino groups on the cell membranes of bacteria. Briefly, dehydrated agents such as carbodiimide EDC and Sulfo-NHS were added to the COOH containing liposome suspension (EDC: NHS: DSPE-PEG-COOH = 30:30:3, molar ratio) in MES buffer (pH=5.5). The mixture was incubated at room temperature for 15 min. The free activating reagents were removed using Sepharose CL-4B column. Then, 100µl of 15 mM activated liposomes were added to bacteria (~10^4 MTB/ml) and incubated for 1 hr at room temperature, under gentle mixing. Liposomes attached to magnetic bacteria were separated from non-attached liposomes by applying a 2D magnetic field. The sample was washed three times in PBS and finally re-suspended in 500 µl of PBS. The following methods were performed to confirm the interaction of liposomes following incubation with bacterial cells:

Fluorescence microscopy

Fluorescent liposomes were prepared to verify the presence of liposomes on the surface of bacteria. The localization of fluorescence dye (TRITC: Ex: Em ~555/580 nm) was observed with fluorescence microscopy using a Zeiss LS510.

Flow cytometry

Bacteria functionalized with TRITC labeled liposome were introduced directly into a flow cytometer (FACS calibur; BD Biosciences) and analyzed. Non-coated bacteria were used as a control.

RESULTS AND DISCUSSION

To achieve a liposome-MTB system with the ability to target tumor cells, the conjugation should be stable and strong. The present study focused on the selection of a suitable method for modification of the liposome surface to allow effective binding to magnetic bacteria. Covalent binding of liposomes to bacteria was evaluated first by fluorescence microscopy, using

TRITC labeled liposome to visualize the attachment of liposomes on the bacteria. Figure 1 clearly shows the successful attachment of liposomes to MTB after 1h incubation.

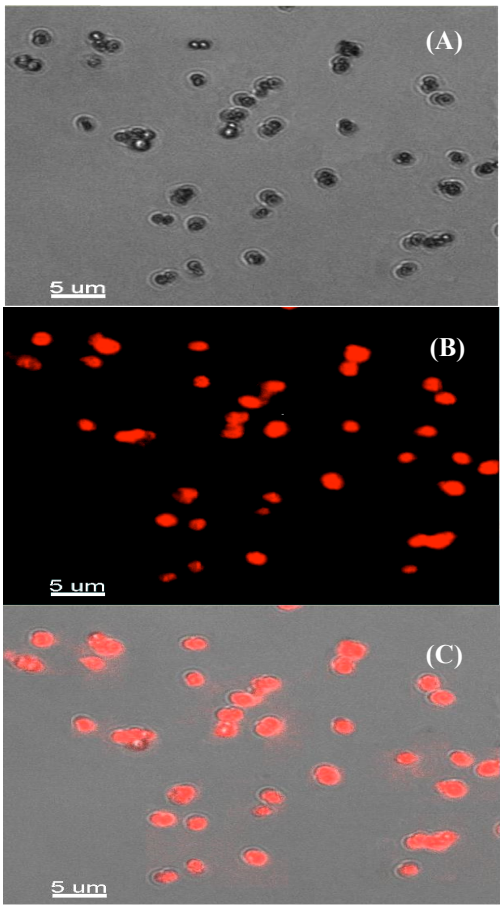

Figure1: Confocal microscopy analysis of TRITC labeled liposome attached to MTB. Photomicrographs show the phase contrast (A), fluorescent (B), and overlay confocal image (C).

Additionally, flow cytometry results demonstrated that all bacteria were successfully coated with liposomes, resulting in a homogenous population of Lip-MTB. As shown in Figure 2, a shift in red fluorescence intensity was observed for MTB-liposome complexes (grey histogram) in comparison to non-coated bacteria (white histogram) indicating the presence of fluorescence liposomes on the surface of bacteria. Approximately 25,000 events were counted for each sample.

CONCLUSIONS

The results demonstrated covalent attachment of carboxylated liposomes to magnetotactic bacterial cell membranes via carbodiimide agents. Ongoing research aims at demonstrating whether this complex could be navigated using an external magnetic field. As such, the complex would be a promising candidate for delivery of active substances to tissues of interest providing an alternative opportunity for the diagnosis and treatment of cancer disease.

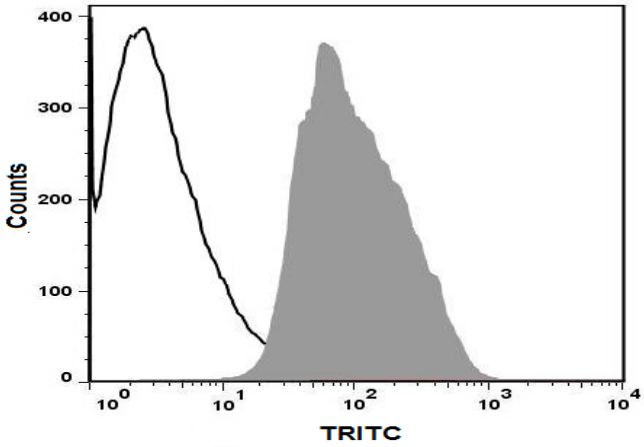

Figure 2: Flow cytometry analysis of non coated bacteria (white histogram) and TRITC labelled liposome pre-incubated with bacteria (grey).

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