Synthetic Filamentous Phages for Anti-Cancer Drug Delivery

Yan Geng and Dennis Discher
Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia 19104

Statement of Purpose: Therapeutic vehicles used to treat pathologies run the gamut from nanoparticles, liposomes to biochemically modified quantum dots.[1-3] However, the vast majority of vehicles studied to date have a *spherical* morphology. Little thought has been given to the importance of vehicle shape concerning circulation times (e.g. biocompatibility/stealthiness). Here, we have prepared flexible *cylindrically* shaped worm micelles self-assembled from biocompatible amphiphilic poly(ε-caprolactone)-*block*-poly(ethylene oxide), PCL-PEO (OCL) copolymers to mimic the micron-long natural filamentous phages and demonstrate the strong effect of vehicle morphology on biological interactions and transport. Interaction of OCL worm micelles with cancer cells was also studied.

Methods: Materials. Diblock copolymers PCL-PEO were purchased from Polymerscource Inc.

Instrumentation. Fluorescent and bright-field images were recorded using an Olympus IX71 inverted microscope with a CCD camera (Cascade). Before injection, OCL worm micelles were repeatedly extruded through 400 nm membrane at 200 psi, to gently reduce the contour lengths of the flexicelles from tens of microns to the desired length, and to eliminate particles that could obstruct capillaries.

In-vivo assays. Sprague-Dawley (SD) male rats were injected with 0.5 ml of 5 mg/ml copolymer in Phosphate Buffered Saline. Orbital bleeds were taken at various times during the study to determine the number, *N*, and contour length of the OCL worm micelles in circulation. Injections, bleeds, and organ harvests were carried out at Covance Research Products Inc. (Denver, PA

Results / Discussion: cylindrical shaped worm micelles were self-assembled from biocompatible OCL diblock copolymers in water, with the weight fraction of PEO, f_{EO}~0.43,[4] by a cosolvent-evaporation method,[5] Fig. 1a. Long worm micelles were observed *in-vitro* to stretch out under flow, minimizing their interactions with phagocytes, Fig. 1B, whereas spherical micelles were phagocytosed by an active white cell in Fig. 1C.

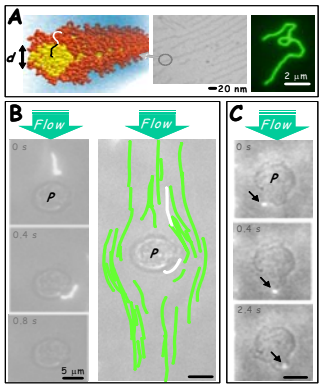


Figure 1. In-vitro phagocytosis of OCL worm micelles and spherical micelles under flow.

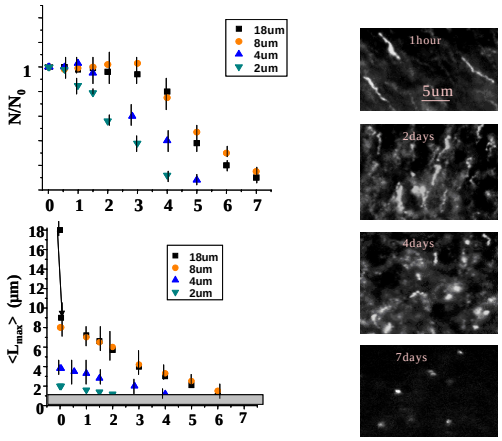
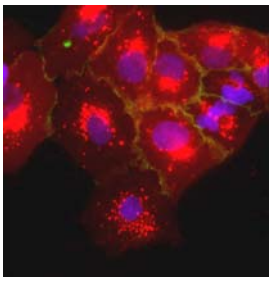


Fig. 2 shows the clearance behavior of flexicelles from rats after tail-vein injection. In contrast to liposomes and polymeric vesicles, which get cleared within one day, the cylindrical worm micelles can circulate up to a week. Worm micelle contour lengths are reduced throughout the course of their circulation in rat vasculature.

Figure 2. Long-circulating OCL worm micelles in rats.



In-vitro incubation of OCL worm micelles with human lung cancer cell, A549, shows that they get internalized with time, Fig.3. Such internalization was temperature dependant, and prohibited by various endoctytosis inhibitors. Internalization of OCL worm micelles by cancer cells demonstrated the ability of OCL worm micelles to deliver encapsulated drugs inside the cell.

Figure 3. Internalization of OCL worm micelles by A549.

Conclusions: As a synthetic mimicry of filamentous phages, flexible cylindrical –shaped worm micelles were self-assembled from OCL diblock copolymers and demonstrated extremely long circulation times. The long circulation times of flexicelles provides a simple cylindrical design scaffold on which to build more specialized delivery systems for treatments of a variety of pathologies, especially for cancer therapy.

References:

(1) Marshall, E. *Science* **2000,** *286*, 2244.
(2) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967.
(3) Pasqualini, R.; Ruoslahti, E. *Nature* **1996**, *380*, 364.
(4) Jain, S.; Bates, F. S. *Science* **2003**, *300*, 460-464.
(5) Geng, Y. ; Discher D.E. JACS **2005**, *127 (37), 12780-12781*