Development of a Heparin-Based Coacervate Loaded Liposomes as Non-Invasive Therapy for Myocardial Infarction

CNF Project Number: 2754-18

Principal Investigator(s): Yadong Wang

User(s): Chia-Wei Yeh

Affiliation(s): Biomedical Engineering, Cornell University

Primary Source(s) of Research Funding: Cornell Startup Funds

Contact: yw839@cornell.edu, cy465@cornell.edu

Primary CNF Tools Used: ABM contact aligner

Abstract:

Cardiovascular disease is one of the major leading causes of death worldwide. Specifically, myocardial infarction (MI), generally known as heart attack, is the main cause of death in cardiovascular disease. Among them, the major cause of death of MI is due to the myocyte necrosis and heart failure. Therefore, it is of particular importance to prevent myocyte necrosis after MI as well as induce infarcted heart tissue to regenerate.

Introduction:

Coacervate is an electrostatically bound complex between cationic and anionic polyelectrolytes. In the extracellular matrix (ECM), glycosaminoglycan such as heparan sulfate proteoglycan (HSPG) binds with several growth factors (GFs) to form HSPG-GF complex. This complex not only serves as reservoir for bonding and stabilization of GFs but also potentiates GFs responsible for maintaining normal cellular function. Due to the similar mechanism of protein-extracellular matrix interaction, it has been shown that heparin-based coacervate is a promising candidate for drug delivery system in biomedical and tissue engineering applications. However, coacervate complex is unstable in the blood stream owing to the relatively weak electrostatic interaction within coacervate droplets, leading to the difficulty to systemically administer coacervate via intravenous injection.

To solve this problem, we aim to encapsulate heparin-based coacervate complex into liposome, namely coacersome, for a non-invasive therapy for MI. In this study, polyanion heparin is utilized to complex with vascular endothelial growth factors C (VEGF-C) to form heparin-growth factor complex, which is then mixed with synthetic polycation, ploy(ethylene arginyl aspartate diglyceride) (PEAD) to construct VEGF-C loaded coacervate droplets. In order to enhance coacervate complex stability in the blood stream, an on-chip microfluidic device is used to generate coacersomes by encapsulating VEGF-C loaded coacervates into liposomes in a well-defined manner. The therapeutic effect of the coacersomes will be evaluated on rat myocardial infarction model.

Summary of Research:

The microfluidic device is designed to generate liposomes encapsulated with coacervate complex in different size by using different flow rate among outer aqueous phase (OA), inner aqueous phase (IA), and lipid carried organic phase (LO), as shown in Figure 1. OA contains 15% (vol/vol) glycerol and 5% (w/v) P188 in water, IA contains 15% (vol/vol) glycerol and 20 μg/mL FITC-heparin water, and LO contains 0.2% (wt/vol) DOPC in 1-octanol. Liposomes are successfully generated via microfluidic device, and the flow rate is 5 μL/min for each phase, as shown in Figure 2. At this time point, the size distribution is wide — the diameter of liposome ranges from 10 to 200 μm.

Next, FITC-heparin is successfully encapsulated into DOPC liposomes, and the flow rate is 0.1 μL/min for each phase, as shown in Figure 3. The diameter of FITC-heparin encapsulated DOPC liposome ranges from few microns to 10 μm. The encapsulation efficiency is 40% to 50% at this time point. In order to generate liposomes in different size, two different flow rate are used to generate DOPC liposomes (0.1 μL/min and 5 μL/min for each phase, as shown in Figure 4), and the diameter is different. However, detailed size distribution is not tested at this time point.

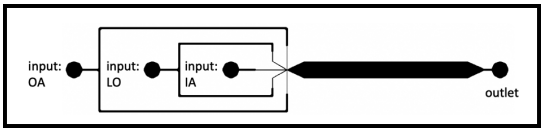

Figure 1: Water-in-oil-in-water double emulsion chip for generating liposome encapsulated with coacervate. OA: outer aqueous phase; IA: inner aqueous phase; LO: lipid carried organic phase.

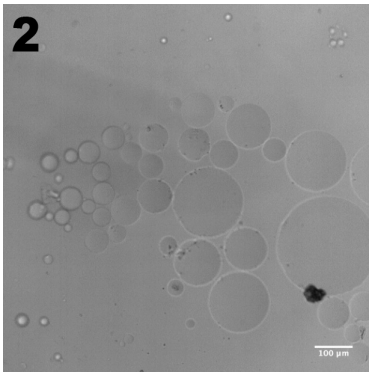

Figure 2: DOPC liposomes generated via microfluidic device. Scale bar: 100 µm.

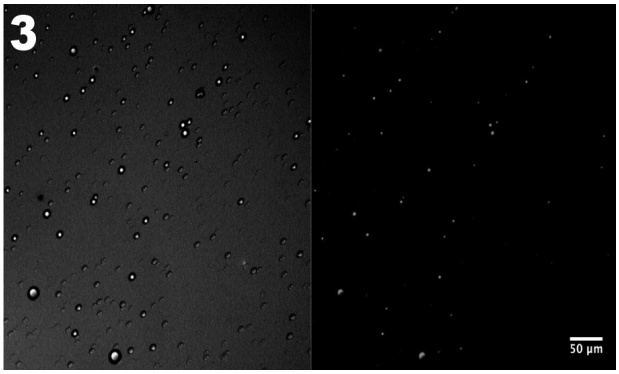

Figure 3: FITC-heparin encapsulated DOPC liposomes, gray scale and fluorescent images. Scale bar: 50 µm.

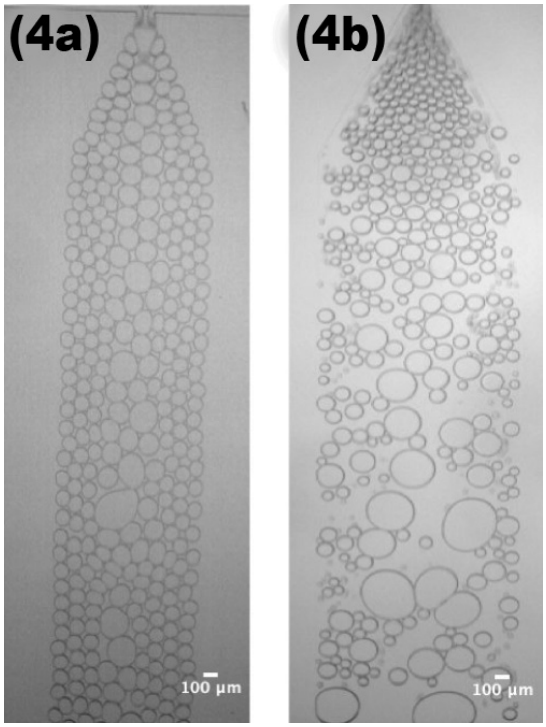

Figure 4: DOPC liposomes generated via microfluidic device. (a) flow rate: 0.1 µL/min for each phase. (b) flow rate: 5 µL/min for each phase. Scale bar: 100 µm.

References:

[1] Deshpande, Siddharth, and Cees Dekker. "On-chip microfluidic production of cell-sized liposomes." Nature protocols 13.5 (2018): 856.

[2] Deshpande, Siddharth, et al. "Spatiotemporal control of coacervate formation within liposomes." Nature communications 10.1 (2019): 1800.