

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

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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.

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 coacervate vesicles or covesicles in short, 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, poly(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 covesicles by encapsulating VEGF-C loaded coacervates into liposomes in a well-defined manner. The therapeutic effect of the covesicles will be evaluated on rat myocardial infarction model.

Summary of Research:

Covesicles are successfully generated in the designed microfluidic chip utilizing three phases: outer aqueous phase (OA), inner aqueous phase (IA), and lipid carried organic phase (LO), as shown in Figure 1 and Figure 2. OA contains 15% (vol/vol) glycerol and 5% (w/v) P188 in water, IA contains 15% (vol/vol) glycerol and PEAD/heparin coacervate complex in water, and LO contains 0.2% (wt/vol) DOPC in 1-octanol. Coacervate complex is formed by mixing PEAD solution and heparin solution prior to flow into microfluidic chip.

From Figure 1 and 2, PEAD/heparin coacervate complex is encapsulated by 1-octanol, forming water-in-oil droplets, then further pinched off by OA solution, and forming water-in-oil-in-water double emulsion droplets at the post-junction area. Moreover, covesicles with uniform size and high encapsulation efficiency are observed under the following flow rate: OA: 900 $\mu\text{L/hr}$, IA: 30 $\mu\text{L/hr}$, LO: 30 $\mu\text{L/hr}$, as shown in Figure 3. The average diameter of covesicles is 17.87 μm .

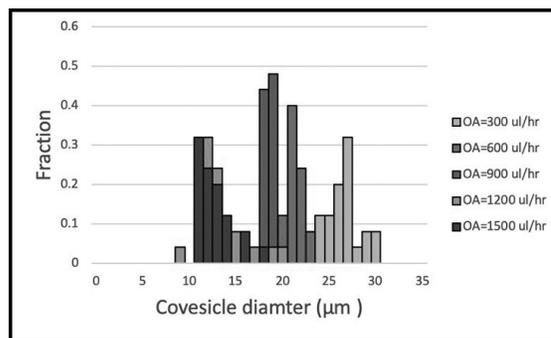
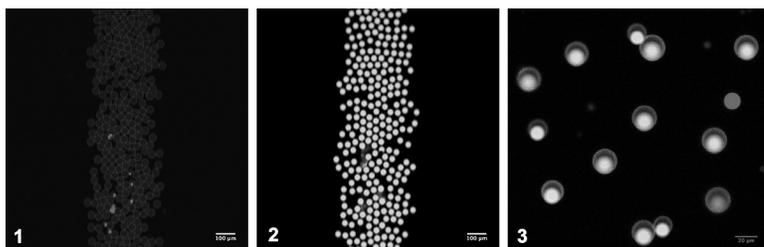


Figure 4: Coesicles diameter distribution vs. OA flow rate.

Figure 1, left: Rhodamine-labeled liposome formed via the designed microfluidic device. Flow rate: OA: 300 µL/hr, IA: 30 µL/hr, LO: 30 µL/hr. Scale bar: 100 µm.
Figure 2, middle: FITC-labeled PEAD/heparin coacervate encapsulated in liposome via the designed microfluidic device. Flow rate: OA: 300 µL/hr, IA: 30 µL/hr, LO: 30 µL/hr. Scale bar: 100 µm.
Figure 3, right: Coesicles with uniform size and high encapsulation efficiency. Red: rhodamine-labeled lipid. Green: FITC-labeled heparin. Flow rate: OA: 900 µL/hr, IA: 30 µL/hr, LO: 30 µL/hr. Scale bar: 100 µm. (See pages vi-vii for full color versions of all three images.)

We are also interested in generating coesicles with various size. Thus, via using different flow rate, the diameter of coesicles ranges from 30 µm to 10 µm is achieved, as shown in Figure 4.

In order to achieve dripping regime during coesicles generation, both of IA and LO flow rate is maintained at 30 µL/hr. As expected, the diameter of coesicles decreases when OA flow rate increases. In our proposed strategy, coesicles less than 10 µm in diameter is our major target.

For next step, we will move forward to encapsulate VEGF-C into coesicles and test the protection effect on VEGF-C.

References:

- [1] Deshpande, S., and C. Dekker. "On-chip microfluidic production of cell-sized liposomes." *Nature protocols* 13.5 (2018): 856.
- [2] Deshpande, S., et al. "Spatiotemporal control of coacervate formation within liposomes." *Nature Com.* 10.1 (2019): 1800.