Development of Heparin-Based Coacervate Loaded Liposomes as 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 myocyte necrosis and heart failure. The acute inflammation after MI may be resolved by draining the excessive tissue fluid through lymphatic networks around the heart. However, the high interstitial pressure in the infarcted area may impede the drainage, and the newly formed lymphatic networks due to the MI are not functional. Thus, unresolved inflammation may further exacerbate the damage to the heart tissue.

Vascular endothelial growth factor type C (VEGF-C) is known for inducing lymphangiogenesis both *in vitro* and *in vivo*. Several studies reported that by administrating VEGF-C after MI on rat model can effectively resolve the acute inflammation, and furthermore improve the cardiac function. Therefore, it is of particular promise to deliver VEGF-C to the infarcted heart area, induce lymphangiogenesis, resolve the acute inflammation after MI as well as facilitate injured heart tissue to regenerate.

Summary of Research:

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 protein delivery system in biomedical and tissue engineering applications. Nevertheless, 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.

In this study, we aim to develop a liposome filled with heparin based coacervate, namely lipocoacervate in short, for protein delivery to treat MI. 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. Also, staggered herringbone micromixer (SHM) microfluidics is designed to generate lipid vesicles. The VEGF-C loaded coacervate will then be mixed with the lipid vesicles to form lipocoacervate. The therapeutic effect of the lipocoacervate will be evaluated on rat myocardial infarction model.

Research Steps:

The negatively charged lipid vesicles were prepared by mixing DOPC/DSPG/cholesterol (molar ratio = 5/1/3) ethanol solution with 0.9% saline in SHM chip. The flow rate ratio between ethanolic and aqueous phase is 5, resulting negatively charged lipid vesicles (zeta potential = -3.11 ± 0.49 mV) with 70 nm in diameter. In order to facilitate the negatively charged lipid vesicles assemble onto coacervate, different PEAD to heparin (P/H) ratio was tested to figure

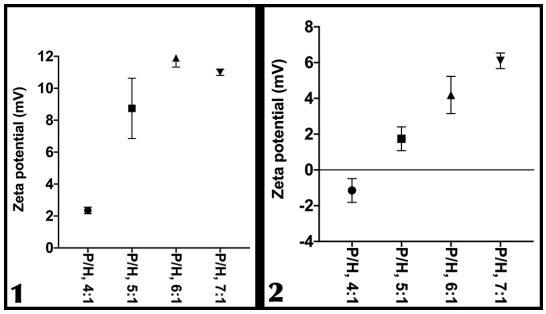

Figure 1, left: Zeta potential of PEAD/heparin coacervate before adding negatively charged lipid vesicles vs. P/H ratio. Figure 2, right: Zeta potential of lipocoacervate vs. P/H ratio.

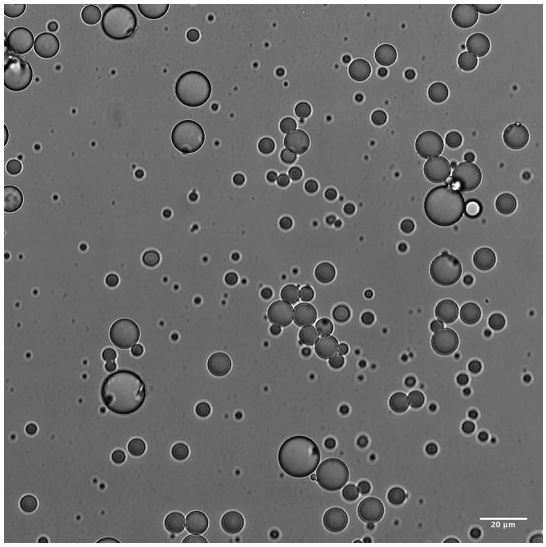

Figure 3: Confocal image of lipocoacervate, bright field. Scale bar, 20 µm.

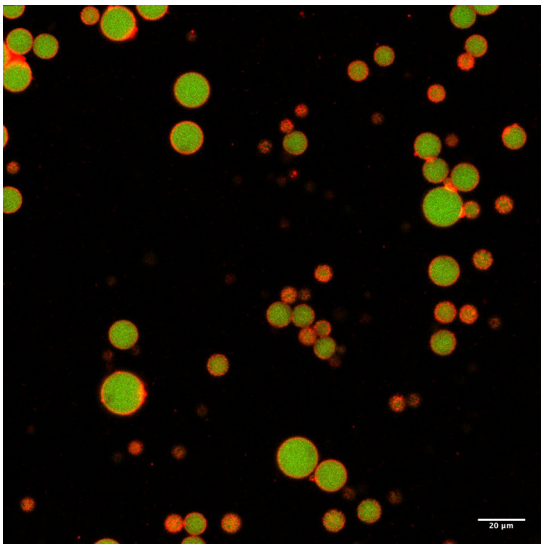

Figure 4: Confocal image of lipocoacervate, red: rhodamine labeled PE; green: fluorescein labeled BSA. The red lipid outside of green coacervate core suggesting the successful assembly of lipocoacervate. Scale bar, 20 µm.

out the zeta potential of coacervate, as shown in Figure 1. As the P/H ratio increased, the zeta potential also increased. When the negatively charged lipid vesicles were mixed with coacervate, the zeta potential was reduced due to the electrostatic adsorption of the negatively charged lipid vesicles on coacervate, as shown in Figure 2. For visualizing the structure of lipocoacervate, rhodamine labeled PE (red) was added into lipid solution during the preparation of lipidvesicles, and fluorescein labeled bovine serum albumin (green) was also incorporated into PEAD/heparin coacervate for visualization.

The confocal images in Figure 3 and Figure 4 showed that the red lipid was indeed outside of green coacervate core, suggesting the successfully assembly of lipocoacervate.

Future works will be focused on investigating the lipid structure outside of coacervate, testing the stability of lipocaocervate, and evaluating the lymphangiogesis efficacy of VEGF-C loaded lipocoacervate.

References:

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