Manipulating endogenous exosome biodistribution for therapy

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Exosomes have been recognized as extracellular vesicles that mediate systemic information exchange and long-distance interactions between cells. Their functions are all highly reliant on systemic biological distribution so that it is possible to promote the biological effects for therapy by accurately manipulating exosome biodistribution. However, given that the mechanisms for regulating the exosome biodistribution are unclear, it is challenging to achieve the manipulation of exosome biodistribution by regulating molecular signals. Recently, Liu et al. reported a vesicle shuttle (VS), which was composed of a ferroferric oxide core, a silica shell, and a stimuli-cleavable poly(ethylene glycol) corona conjugated to two types of antibody (one against antigens on the exosomes of interest, and the other targeted to the recipient injured cells). They showed that the VS could effectively collect, transport, and release circulating exosomes to the designated areas inside the organism.

Previous studies have proved that circulating exosomes can improve heart function after myocardial infarction (MI). Researchers verified the potential of the VS in MI therapy (Figure 1). They showed that the VS has the ability to efficiently increase the concentration of therapeutic circulating exosomes around infarct areas as needed. This enhanced the angiogenesis in the heart tissue and a notable improvement in heart function after MI, showing—at the proof-of-concept level—that it is feasible to manipulate the exosome biodistribution by nanoparticles for therapy.

In the multifunctional VS system constructed by the researchers, each component performs its own functions. The ferroferric oxide core enables the accumulation of VS to injured cardiomyocytes under a local magnetic field. The silica shell not only protects the magnetic properties of the ferroferric oxide core from it leaching in acidic biological environments, but also enables further step-wise modification. The poly(ethylene glycol) modification helps to limit the nonspecific interactions between VS and blood components, thereby prolonging the circulation time of VS in vivo. The pH-cleavable hydrazone bonds can be cleaved under the acidic pH of injured cardiomyocytes to release the captured circulating exosomes. The anti-CD63 against antigens on the exosomes and anti-MLC directed to injured cardiomyocytes in the MI region have the functions of specifically capturing circulating exosomes and binding to injured cardiomyocytes, respectively.

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Researchers first confirmed the therapeutic function of circulating serum exosomes collected from MI rat model. Exosomes isolated from MI rat serum (MI-EXO), normal rat serum (Con-EXO), or phosphate-buffered saline (PBS) were injected into the myocardium of MI-model rats. Two weeks after therapy, the infarct was significantly reduced and the left ventricular ejection fraction was significantly improved in MI-EXO-treated rats but not in Con-EXO-treated and PBS-treated rats. In the ischaemic myocardium region, the area of blood vessels in the MI-EXO group was significantly larger than that in Con-EXO group and PBS group. These results showed that MI-EXO could improve the heart functions of MI model.

Then, the in vitro exosome capture ability of VS was tested. Transmission electron microscopic analysis, fluorescence confocal microscopic analysis, and Western blot assays confirmed that VS could capture circulating exosomes as designed. Furthermore, the researchers indicated that the VS comprising 20 wt% anti-CD63 and 40 wt% anti-MLC antibodies would have the greatest ability to capture exosomes with minimum impact on cardiomyocyte binding.

Before in vivo administration for the manipulation of endogenous exosomes, the in vivo clearance behavior of VS was investigated. The blood elimination half-life of VS was about 3 h, which was sufficient for the targeted enrichment of VS to the MI region under an external magnetic field. The majority of injected VS could be excreted from the body within 60 h through renal clearance and hepatobiliary pathway. The rapid clearance of VS is of great value to minimize systematic toxicity.

Following this, the magnetic-antibody dual-targeting ability of VS in MI-model rats and minipigs was studied. Nanoparticles with anti-CD63 and anti-MLC antibodies replaced by isotype control antibodies were served as a control group. The ex vivo imaging and fluorescence confocal microscopy analysis showed that the application of a magnetic field could significantly increase the control group content in the heart. Without applying a magnetic field, confocal fluorescence microscopy analysis showed that VS significantly increased the number of exosomes that were adjacent to the injured cardiomyocytes, whereas the control group neither bound to injured cardiomyocytes nor engaged with exosomes, indicating that the binding effects of the dual antibodies conjugated VS were specific.

The exosome release of VS in the simulated infarct environment and infarcted area of rats was also studied. The anti-release behavior of VS under simulated physiological conditions (pH 6.8 as an infarct environment and

![Figure 1](attachment:vesicle_shuttle.Diagram.png)  
**Figure 1** Schematic of the vesicle shuttle (VS) approach. (i) The anti-CD63 antibodies conjugated to the VS capture and attach to exosomes. (ii) Dual targeting of the VS using both an external magnet, which attracts the magnetic VS to the infarct area, and a conjugated anti-MLC antibody, which targets MLC on damaged cardiomyocytes. (iii) Release of the exosomes triggered by the infarct environment of pH <6.8, acidosis-induced cleavage of hydrazone bonds, and shedding of the VS corona lead to the selective release of exosomes. Reproduced from Liu et al."
PH 7.4 as a normal environment) was evaluated. After incubating for 12 h in medium, only 19% of antibodies were released at pH 7.4 medium while it was up to 95% at pH 6.8. This confirmed that pH played an important role in triggering the exosome and antibody release from VS. For VS combined with exosomes, after incubating in PBS at 37°C for 4 h, only a few exosomes were released at pH 7.4 while most exosomes were released at pH 6.8. Furthermore, the in vivo exosome-release behavior of VS was also confirmed by fluorescence imaging.

Finally, VS was administered systemically in MI model rats and rabbits to determine the therapeutic effects. After 4 weeks of therapy, VS therapy significantly reduced MI size, improved the left ventricle ejection fraction, and boosted angiogenesis compared with control groups. The blood chemistry and histological examination of major organs after therapy revealed no apparent lesion evidence of toxicity. On the whole, VS could be applied as a safe formulation for endogenous exosome manipulation and therapy.

The concept of manipulation of endogenous exosome biodistribution for therapy of MI could be extended to other diseases. Furthermore, the manipulation of exosomes could be leveraged to study the biological roles of their biodistribution and to manipulate the biodistribution of other extracellular vesicles, such as microvesicles and apoptotic bodies, which also play important roles in signal transformation, cell function regulation, and the maintenance of tissue homeostasis.5,6

Despite the above results and expansion potential, as the authors have pointed out, considering the effect of different pathological microenvironments at different disease stages on the transferred exosomes and on the functions of VS, further study on optimal usage of VS for practical therapy application is required. Besides, finding disease-specific exosome markers and corresponding antibodies may further enhance the effect of VS.

CONFLICT OF INTEREST
The authors declare no conflict of interest. [Correction added on 29 June 2021, after first online publication: Conflict of Interest section has been added.]

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Zhiwei Sun is currently a Ph.D. student in the School of Materials Science and Engineering at Shandong University under the supervision of Prof. Yanyan Jiang. His research interest lies in the development of biosensing platforms for detecting clinically relevant exosomes in various diseases. Prior to starting his PhD, he received his B.E. (2016) and M.E. degree (2019) from Qingdao University of Science and Technology and Hebei University of Technology, respectively.

Yanyan Jiang completed her PhD in Prof. Martina Stenzel group, School of Chemical Engineering at the University of New South Wales, Australia in 2016. She was awarded (2016) Japan Society for Promotion of Science (JSPS) Post-doctoral Research Fellowship at Kyoto University under the supervision of Prof. Itaru Hamachi. Since 2018, she has been appointed as a full professor of materials science and engineering at Shandong University. Her research interests are in the synthesis of functional nanoparticles serving as anticancer drug carriers, biosensors, catalysts, and theoretical studies of the mechanism and properties of these nanoparticles.

Martina Stenzel studied chemistry at the University of Bayreuth, Germany, before completing her PhD in 1999 at the University of Stuttgart, Germany. She started as a postdoctoral fellow at UNSW in 2000 and is now a full Professor. Her research interest is focused on the synthesis of
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