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CARDIOPULSE

Global Spotlights

Small blebs, big potential — can extracellular vesicles cure cardiovascular disease?

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If we have learned anything during the current COVID-19 pandemic, it is the importance of effective delivery systems for messages and goods. Social media, virtual meetings, and home-delivered food have helped us endure the hardship of various stages of isolation and quarantine, across Europe and the world. In our streets and cities, the available delivery systems differ in their speed, volume, cargo security, and cost. In our bodies, there are also a number of systems for the delivery of information and cargo. Some are fast and goal-directed but also consume a lot of energy and are strictly hierarchical, such as the nervous system. Others, such as extracellular vesicles (EVs), are open for use by every cell, broad in potential applications, and 'inexpensive' but still protective of their cargo.

EVs were first discovered to act as the workhorses of transport in the body about the same time that Sting released his No. 1 hit with the appropriate title 'message in a bottle'. Peter Wolf discovered what he called 'platelet dust', which could accelerate coagulation *in vitro*.[1] Since then, considerable progress has been made in the field and EVs have been shown to be involved in various pathological processes including inflammation, calcification and apoptosis. EVs do not only transport by-products or 'waste' but are drivers of the aforementioned processes and therefore interesting therapeutic targets.

When the cargo-delivery function of EVs was first discovered for thrombosis and later for atherosclerotic disease,[2] there was much enthusiasm in the field. Understanding the mechanism by which they work seemed to offer a 'field of gold' of new therapeutic opportunities with the potential to re-'shape people's hearts'. However, there are still no EV-based therapeutics, owing in part to difficulties in manufacturing them, which are only now close to being overcome.

Unlike small-molecule drugs, which are produced routinely, EVs are complex biological structures that require a lipid membrane to encapsulate their cargo as well as specific proteins to mediate their uptake into somatic cells. Therefore, the manufacturing process is complicated and not easily scalable, but there seem to be two promising methods to begin producing large amounts of EVs (*Figure 1*).

First, proliferating human cells can be used to generate authentic EVs. This requires bioreactors, where mostly mesenchymal stem cells (MSC) are cultured for the production of EVs. While this method is relatively costly, it has the advantage of producing authentic biologically derived EVs, which are believed to be less immunogenic and more effective for uptake and cargo delivery than the alternative approaches.

A second, promising method is the extraction of xenogenic EVs from plants or whey, which can be used to deliver drugs as well as siRNAs.[3] The big advantage here is the low cost of production and the large amount of EVs that can be produced. Therefore, less effective uptake of these vesicles can be compensated for with a higher dose.

Furthermore, EV-like structures, called lipid nanoparticles, can be manufactured synthetically. These are small, synthetic vesicles, which are technically not EVs that can also be used to effectively transfer biomolecules into cells. An interesting example of this approach is the vaccines against SARS-CoV-2 from Biontech/Pfizer and Moderna, both of which rely on the use of lipid nanoparticles to deliver mRNA. This procedure seems to be safe and adaptable to other, for example, cardiovascular applications. One of the many imaginable applications for this approach is the local or systemic delivery of pro-regenerative RNAs in lipid nanoparticles after an acute ischaemic event.

In spite of the current technical issues with EV manufacturing, cargo manipulation, and uptake, there are currently >30 interventional studies registered on clinicaltrials.gov, which are evaluating EVs as therapeutic agents.

Most of these studies use EVs from mesenchymal stem cells, because EVs are thought to be responsible for many of the beneficial effects of stem cells without the drawbacks of uncontrolled proliferation and an anti-cellular immune response. Systemic intravenous application of EVs is being tested for multi-organ failure, type I diabetes mellitus, and lung cancer, but there are also studies testing local administration of EVs for peptic ulcers, colon cancer, or COVID-19 pneumonia.

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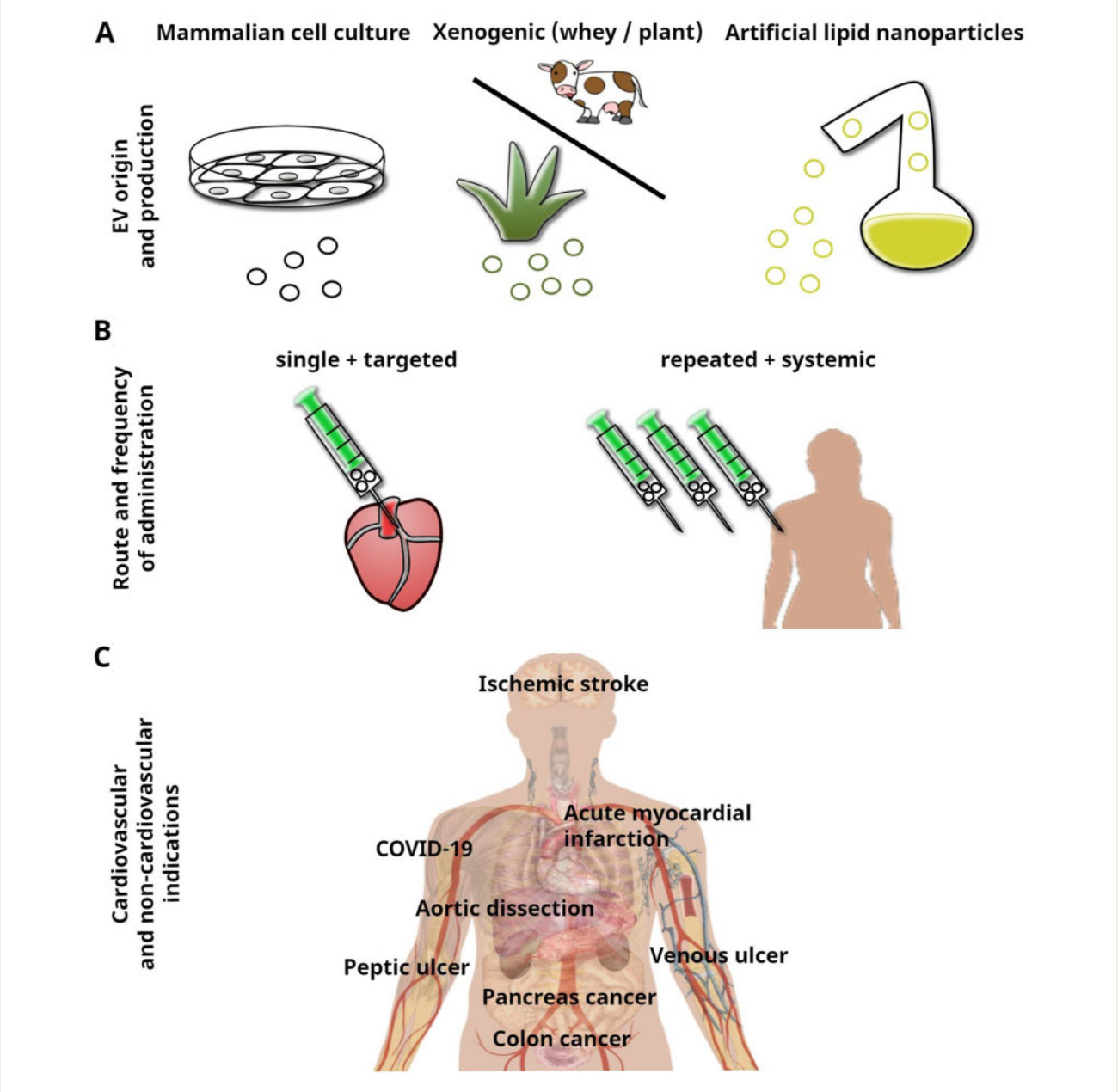

Figure 1 Summary of current trends in extracellular vesicle therapeutics. (*A*) Origin and production of extracellular vesicles. (*B*) Single vs. repeated and local vs. systemic administration. (*C*) Currently investigated cardiovascular and non-cardiovascular indications for extracellular vesicle treatments.

In the cardiovascular field, pre-clinical trials with EVs have been performed now for more than a decade.[4] Recently, the coating of stents with EVs resulted in accelerated re-endothelialization and reduced in-stent re-stenosis compared to drug-eluting or bare-metal stents in mice.[5] This innovative approach gives cardiologists hope to overcome the limitations of drug-eluting stents and has important clinical implications for patients with coronary artery disease.

Another highly interesting study will test the effect of intracoronary applications of EVs after myocardial infarction (NCT04327635). Local intracoronary administration may be particularly beneficial in an acute setting, because it avoids two of the biggest problems of EV therapeutics: achieving a sufficiently high systemic concentration and assuring that EVs are delivered to the site of interest. Furthermore, the effect of EVs on organ dysfunction after surgical repair of Stanford A aortic dissections is currently being investigated. Here, the EVs will be applied intravenously daily for 14 days (NCT04356300). In contrast to the aforementioned study, this trial will therefore test the effectiveness of systemic, repeated administration. These two studies are at the ends of a wide spectrum of therapeutic EV approaches, ranging from targeted single use to repeated systemic administration (*Table 1*).

The good news is that the first interventional clinical trials with EVs in the cardiovascular field have begun, attempting to deliver individualized cargo at a precise time and place, allowing cardiologists for the first time to build a 'fortress around your heart'. In our opinion, this is a

Table 1 Current clinical trials of extracellular vesicle-based therapeutics for cardiovascular diseases

Condition	Origin of EVs	Administration	NCT number	Study start
Multiple organ failure after surgical repair of Stanford type A aortic dissection	Exosomes from MSCs	150 mg i.v. 1×/d for 14 days	NCT04356300	1 September 2020
Acute myocardial infarction	Purified exosome product (PEP)	5%, 10%, or 20% PEP intracoronary 1× after PCI	NCT04327635	26 April 2021
Venous ulcer	Autologous extracellular vesicles from serum	Peri-wound injection 1×/week for 3 weeks	NCT04652531	18 September 2020
Ischaemic stroke	Exosomes from MSCs after transfection with miR-124	1×, 1 month after stroke, via stereotaxis/intraparenchymal	NCT03384433	17 April 2019

development that merits close attention, because it can either lead to the realization of potent new treatment options or to the rapid demise of the rising star of EVs.

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Conflict of interest: none declared.

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