HHS Public Access

Author manuscript

Curr Stem Cell Res Ther. Author manuscript; available in PMC 2021 January 13.

Published in final edited form as:

Curr Stem Cell Res Ther. 2020 ; 15(8): 674–684. doi:10.2174/1574888X15666200309143924.

Exosome and Biomimetic Nanoparticle Therapies for Cardiac Regenerative Medicine

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Abstract

Exosomes and biomimetic nanoparticles have great potential to develop into a wide-scale therapeutic platform within the regenerative medicine industry. Exosomes, a subgroup of EVs with diameter ranging from 30–100 nm, have recently gained attention as an innovative approach for the treatment of various diseases, including heart disease. Their beneficial factors and regenerative properties can be contrasted with various cell types. Various biomimetic nanoparticles have also emerged as a unique platform in regenerative medicine. Biomimetic nanoparticles are a drug delivery platform, which have the ability to contain both biological and fabricated components to improve therapeutic efficiency and targeting. The novelty of these platforms holds promise for future clinical translation upon further investigation. In order for both exosome therapeutics and biomimetic nanoparticles to translate into large-scale clinical treatment, numerous factors must first be considered and improved. Standardization of different protocols, from exosome isolation to storage conditions, must be optimized to ensure batches are pure. Standardization is also important to ensure no variability in this process across studies, thus making it easier to interpret data across different disease models and treatments. Expansion of clinical trials incorporating both biomimetic nanoparticles and exosomes will require a standardization of fabrication and isolation techniques, as well as stricter regulations to ensure reproducibility across various studies and disease models. This review will summarize current research on exosome therapeutics and the application of biomimetic nanoparticles in cardiac regenerative medicine, as well as applications for exosome expansion and delivery on a large clinical scale.

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CONFLICT OF INTEREST

K.C. is an equity holder of Xollent Biotech. Xollent provides no funding to the present study.

Keywords

Exosomes; regenerative medicine; biomimetic nanoparticles; drug delivery; extracellular vesicles; mesenchymal stem cells; secretome

1. INTRODUCTION

With heart diseases being a leading cause of death in the United States, advancements in cardiac regenerative medicine have accelerated in the past few years [1, 2]. A large issue in recovering from myocardial infarction (MI) is the restoration and proliferation of cardiomyocytes to the damaged heart tissue [3]. Non-cellular options to treat heart disease are becoming increasingly favorable, as drawbacks such as tumorigenicity and immunogenicity are prevalent in cell-based therapies. There have been multiple reports of tumor formation at the site of transplantation of stem cells [4, 5]. Additionally, immune rejection of stem cells is a factor that remains somewhat unpredictable when stem cell therapies are administered [6, 7]. Both of these risks also contribute to the overall variability that comes with cellular therapies, making it more difficult to translate these therapies clinically [8].

Extracellular vesicles (EVs) have become a promising topic for cardiac regenerative medicine because of their ability to serve as a protective transportation system between cells [9]. EVs are membrane-bound structures released from cells and contain various types of cargo, depending on the individual cell origin and its surrounding environment [9]. EVs are released from most cell types and can be isolated from almost all biological fluids and media they are cultured in ex vivo [10]. They are known to carry proteins, lipids, DNA, mRNAs, and non-coding RNAs [9]. EVs can be further subdivided based on their size and biogenesis methods [9]. Although EVs have become an attractive option for researchers, much is still unknown and unclear about their specific characteristics and how they might fit in the topic of regenerative medicine. There have been reassessments of the past work by Orekhov *et al.* on extracellular vesicles as well as new studies on the *in vitro* effects of certain drugs on cells from atherosclerosis patients' lipid uptake [11]. One specific subpopulation of EVs, exosomes, has become a novel concept in cardiac regenerative medicine.

Another concept that has proven therapeutic efficiency is biomimetic nanoparticles. These synthetic nanoparticles recreate naturally occurring interactions between cells in the body and use cell imitation to effectively deliver a therapeutic agent [12]. Biomimetic nanoparticle platforms are advantageous in the sense that they are able to combine both synthetic and natural materials to create a more targeted drug delivery method [13]. They also offer a more prolonged release of therapeutic factors in comparison to other platforms and are biodegradable in the body [14]. Application of nanoparticles in drug delivery towards cancer treatment, as well as cardiovascular disease treatment have been explored [15, 16]. For example, gold nanoparticles have recently begun studies in early clinical trial phases [17]. These nanoparticles are able to localize tumors, making it easier to then expose the tumor to an excitation source for treatment [17]. Application of nanoparticles in cardiac regenerative medicine has also emerged, as these particles are able to effectively delivery therapeutic

components to treat various heart diseases. In numerous studies, unique platforms revolving biomimetic nanoparticles as a method for drug delivery yielded favorable results in the treatment of cardiac injuries, such as ischemia/reperfusion (I/R) injuries and MI [18, 19]. Both biomimetic nanoparticles and exosomes offer novel platforms for the treatment of cardiac diseases, thus, it is important to continue expanding our knowledge of these topics.

1.1. Exosomes for Drug Delivery Purposes

Exosomes are a subgroup of EVs with their sizes typically ranging from 30–100nm [20]. Exosomes are released into the extracellular space from multivesicular bodies (MVBs) [21]. They undergo invagination during formation, giving them the same membrane complex as the plasma membrane, allowing for an effective transfer of information between cells [21]. Exosomes, therefore, have intrinsic characteristics, making them less likely to have immune rejection responses when used clinically [22]. When MVBs fuse with the plasma membrane, exosomes are either released from the cells or are fused with lysosomes. When fused with lysosomes, exosomal content is degraded instead of secreted [21]. Numerous factors are considered before the intercellular decision to fuse with the plasma membrane or the lysosomes. MVBs' interactions with actin, as well as the microtubule cytoskeleton, have been shown to affect their fusion with the plasma membrane [23]. Cortactin (an actin binding protein) levels have also been shown to affect whether exosomes are secreted or degraded [24]. Studies have been carried out to explore ways to control and manipulate the cellular decision to either secrete or degrade the exosomes [25]. Exosome secretion has been reported to increase under hypoxic conditions, as well as under cellular stress [26, 27]. Knowing these factors that increase exosome secretion can open future pathways for exosome therapeutics.

Many of exosomes' characteristics make them an ideal drug delivery vehicle. The fact that exosomes are found in all biological fluids shows their tolerance in the body, which increases the likelihood that exosome drug delivery vehicles will also be tolerated [22]. Another beneficial characteristic is their ability to be loaded with various cargos for therapeutic effects. Exosomes can be loaded with therapeutic agents before or after they are isolated from biological fluids [28]. Pasucci *et al.*, loaded Paclitaxel (PTX) into mesenchymal stem cell (MSC)-derived exosomes by incorporating PTX into the incubation of the MSC cells. After isolating the PTX loaded MSC-derived exosomes, they were shown to exhibit anti-tumor activity [29]. In another study, Haney *et al.*, evaluated four different methods of loading exosomes after they were isolated. Haney incorporated catalase into exosomes through an incubation process with and without a saponin treatment, freeze-thaw cycles, sonication, and extrusion. The results from Haney's study showed that the most effective catalase loaded exosomes for drug delivery were those obtained from sonication, extrusion, and incubation with saponin [30]. Another characteristic which makes exosomes great drug delivery vehicles is their ability to non-invasively cross the blood brain barrier (BBB) [20]. The BBB restricts large molecule drug delivery, as well as 98% of small molecule drug delivery, which poses an issue for many therapeutic platforms [31]. However, exosomes have been shown in multiple studies to cross the BBB and deliver a therapeutic payload, making them an advantageous platform in regenerative medicine as well as treatment of neurological disorders [32, 33]. All of these factors make exosomes an ideal

vehicle for drug delivery for many applications, including drug delivery in the treatment of various diseases including heart disease.

In order for exosomes to remain an ideal candidate for drug delivery, it is essential they are efficiently isolated from different complexes in the body and purified. Over the past few years, these isolation techniques have been explored and refined for different purposes of exosome isolation [34]. Size based exosome isolation techniques, such as ultrafiltration and size exclusion chromatography (SEC), offer a quick method of isolating the exosomes [34]. In these techniques, little pressure or force is applied to separate the exosomes as is with other methods, instead a membrane is used for the separation process [35]. This option usually yields larger amounts of exosomes; however, it can be difficult to purify the end products [35]. An alternative method of isolation, such as immunoaffinity capture, isolates high purity exosomes from cell-free samples. However, this technique produces relatively low yields and is only applicable for cell-free samples [34]. Exosome precipitation alters the exosomal sample by adding a precipitation reagent to capture and isolate exosomes within a certain size range [35]. This technique offers promising future integration for clinical usage because of its straightforward protocol and its ability to collect uniform exosome yields. However, in this technique, the exosome isolation yield is often contaminated with other isolated materials and most precipitation reagents are not cost effective [35]. Ultracentrifugation is another exosomal isolation technique which separates the particles in a sample containing exosomes by density [34]. One survey cites this technique to be the most widely used primary isolation technique across researchers in thirty countries [36]. Although this technique is cost effective and reliable, exosomes are often lost or damaged in this process, due to the intensity of high-speed centrifugation [34]. Lastly, microfluidics-based exosome isolation techniques are a newer approach that traps exosomes through a microfluidic device, while simultaneously filtering out non-exosomal debris [34]. The setbacks of this technique include a low sample capacity and lack of indepth exploration on the protocol [34].

In summary, there are various modes of exosome isolation, all with unique advantages and disadvantages. Since there is no uniform protocol for exosome isolation, the chosen procedure usually varies among studies. This can make comparing exosome data difficult, as using different isolation techniques may lead to a variation in exosome yields and quality between studies.

1.2. Microvesicles for Drug Delivery Purposes

Microvesicles have also emerged as an innovative approach to disease treatment in regenerative medicine. Microvesicles (MVs) are another subgroup of EVs, with their sizes typically ranging from 100–1000nm [37]. MVs are released from the direct budding of membrane vesicles to the plasma membrane [38]. Unlike the secretion of exosomes, the release of MVs is in response to stimuli [9]. Stimuli that have an effect on the releasing of MVs include but are not limited to cytokines, hypoxia, and thrombin [39]. Physical conditions such as MI, obesity, and diabetes can also trigger the release of MVs [39]. As with exosomes, scientists have been able to somewhat manipulate the secretion rates and

times for MVs [40]. In one study, the deregulation of membrane-associated enzymes, called neutral sphingomyelinase (SMPD2/3), showed an increase in MV secretion [40].

Because of microvesicles' inducible traits, their cargo can also vary depending on the stimuli initiating their secretion [9]. MVs cargo can differ even when they are released from the same stimuli, due to intrinsic dissimilarity of different cell types [39]. Various biomarkers for cardiometabolic diseases are also thought to be carried by MVs. Although there are still several limitations of MVs as biomarkers, including the standardization of protocols and the exploration of MVs carried by various other molecules, time and efforts should be put forth into exploring them as this could lead to new insights in the treatment of cardiometabolic disease [39]. These characteristics make MVs a novel platform for regenerative medicine and drug delivery.

Additionally, MVs open pathways for therapeutic platforms, as they have shown to be involved in angiogenesis [41]. Incubating human umbilical vein endothelial cells (HUVECs) with MVs has been shown to indicate the role of angiogenesis through the formation of stable, branched networks [42]. Increased circulation of MVs with various diseases, including cardiovascular diseases, along with their angiogenic effects make them a great candidate for future regenerative medicine therapies.

2. CARDIAC REGENERATIVE MEDICINE

Cardiac progenitor cells (CPCs) have been a popular cell type for the treatment of cardiovascular disease [43, 44]. CPCs are found in different areas of cardiac tissue and have the ability to differentiate into various cardiac cell types, such as myocytes or endothelial cells [45]. CPCs also have the ability to be cultured and expanded from various myocardial tissues, such as human, pig, mouse, and rat [46]. Their therapeutic effects on the treatment of acute MI have also been studied by Assmus *et al.* CPCs have been shown to enhance neovascularization as well as ventricular function, both important aspects of healing from MI [47].

Pluripotent stem cells also offer promising aspects to tissue engineering because of their abilities shown in murine hearts to migrate, differentiate and help rehabilitate heart cells post trauma [48]. One study from Lu *et al.*, showed how induced pluripotent stem cells (iPSCs) derived from multipotential progenitor cells (MPCs) were used to repopulate a decellularized mouse heart. These MPCs were able to differentiate into various cell types, to fulfill the unique role each cell plays in the heart [48]. By using different growth factors, the rate of the new cardiac muscle formation was also able to be controlled throughout the rehabilitation process [48]. Although these benefits of iPSCs have been studied only in murine hearts, this platform gives ways to future possibilities in tissue engineering, as well as a way to better understand the developmental stages of the different layers of the heart [48]. As more studies are carried out, hopefully similar beneficial outcomes with iPSCs can be seen in human hearts as have been seen in murine hearts.

Mesenchymal stem cells (MSCs) have gained popularity and ethical credibility in cardiac regenerative research. Their immunosuppressive and anti-inflammatory capabilities have

made them possible candidates as therapeutic agents in treating a variety of diseases, including myocardial infarction (MI) [49, 50]. Another important aspect of MSCs includes their paracrine effect [51, 52]. This effect has been shown to protect cardiomyocytes *in vitro* [51]. These characteristics, along with many others still being explored, make MSCs interesting for tissue regeneration and repair. They can be obtained from various different sources, including bone marrow, placental or adipose tissues, and umbilical cord [50]. MSCs have also been shown to release large amounts of EVs, which have been shown to induce anti-inflammatory cytokines [50].

Although the cardiac benefits of these cell types have been studied time to time, patient rejection and tumorigenic effects are still setbacks with these treatments [45]. However, many of these cell types have not been deemed “useless” for the treatment of heart disease. Other ways to incorporate these cells in the treatment of cardiovascular disease have emerged, such as patches and hydrogels. Several studies have incorporated cardiac stem/stromal cells (CSCs) into various patch designs to treat MI in a rat model [53, 54]. One study explored encapsulating human CSCs in a thermosensitive nanogel for the treatment of MI in mouse and pig models [55]. Another study reported a synthetic thermosensitive injectable micro- gel platform for tissue regeneration in a mouse model post MI [56]. Magnetic stem cell targeting has also emerged to help combat low cell retention rates as well as other issues commonly found with stem cell therapies [57]. While these platforms have proven beneficial, scientists have also started exploring the unique benefits of exosomal secretions from these cell types and how their exosomes may play a role in future regenerative medicine.

2.1. Mesenchymal Stem Cell-Derived Exosomes

Because of their cell-free aspect, as well as other therapeutic benefits, mesenchymal stem cell-derived exosomes have gained great popularity to with researchers over the past few years. *In vitro* studies on rodents have shown the benefits of MSC-derived exosomes on recovery from MI. In a study by Wang *et al.*, adult MSC-derived exosomes were shown to improve angiogenesis and cardiac function after being injected into mice post-MI [41]. These exosomes have also been shown to stimulate neovascularization and anti-inflammation in rats after ischemic injury [58]. Huang *et al.*, also performed a study where MSC-derived exosomes were shown to have pro-cardioprotective effects in a rat model [59]. All of these factors are crucial in repairing the microenvironment from ischemia, highlighting the potential of MSC-derived exosomes in cardiac regenerative medicine.

In another study by Ju *et al.*, cardiac mesenchymal stem cells (C-MSCs) were isolated from adult mice and exosomes derived from these C-MSCs were obtained through the culture medium. The C-MSC-derived exosomes were purified and injected into mice that were previously subjected to myocardial infarction, to assess the long-term effects of the exosomes on cardiac repair after ischemia [60]. The results of this study concluded that the C-MSC-derived exosomes slowed the deterioration of left ventricular function one month after myocardial infarction [60]. Additionally, Ju observed that the hearts treated with the C-MSC-derived exosomes contained a higher density of capillaries and proliferative cells post

MI [60]. This is just one of many studies to show the angiogenesis promoting characteristics of exosomes derived from MSCs and their potential clinical uses to treat heart disease.

2.2. Exosomes from the Blood

In the past, stem cells derived from human umbilical cord blood (UCB) have been widely explored because of their low risks to donors for disease, as well as their ease in accessibility [61]. However, using UCB-derived stem cells entails the same tumorigenic risk factors as other cell-based therapies, making their applicability constrained [62]. Exploration of exosomes derived from UCB has increased in popularity over the past few years. Because of the wide variety of benefits UCB already provides, these exosomes provide great alternatives to cell-based therapies for future cardiac regenerative medicine applications [62, 63].

One study by Luo *et al.*, compared angiogenesis factors in pigs with intrauterine growth restriction (IUGR) and normal pigs by examining UCB exosomes from both types of pigs. UCB exosomes from IUGR pigs were hypothesized to contribute to abnormal vascular development [63]. Luo *et al.*, found that UCB exosomes from normal pigs enhanced cell growth and promoted HUVEC migration *in vitro*, suggesting that these exosomes have pro-angiogenesis effects. Mean-while, UCB exosomes from pigs with IUGR were shown to have anti-angiogenesis effects [63]. It was also found that miR-150 in UCB exosomes may be a key regulator for angiogenesis in utero as shown through enhanced proliferation and migration of HUVECs when miR-150s were introduced [63]. The angiogenic characteristics of UCB-derived exosomes as well as their miRNA levels within may provide novel approaches for cardiac therapeutic methods.

In another study by Hu *et al.*, mice with cutaneous wounds were injected with UCB exosomes to determine their healing benefits and to further explore their angiogenic properties. Mice injected with UCB exosomes experienced an accelerated healing process, versus mice with the controlled (PBS) injection [62] Additionally, the experimental mice group showed a larger number of newly formed blood vessels at the site of the wound, suggesting that UCB exosomes have angiogenesis enhancing qualities [62]. This study also focused on the reasoning for UCB exosomes' pro-angiogenic effects by focusing on miRNAs [62]. Hu *et al.*, found that miR-21–3p (previously known to enhance endothelial cell angiogenesis) was the most highly expressed in the exosomes. UCB exosomes were observed to have the ability to transfer miR-21–3p to neighboring fibroblast and endothelial cells to promote angiogenesis and thus accelerate the wound healing and regeneration process [62]. As you can see from the following examples, exosomes' ability to travel through the blood allows for drug delivery to occur any-where in the body, giving great potential for treatment and tissue regeneration.

Exosomes' ability to travel through the blood can be beneficial in many ways. In one way how blood-derived exosomes have been advantageous is in tracking treatment efficacy in neurodegenerative diseases [64]. In early stages of neurodegenerative diseases such as Alzheimer's and Parkinson's disease, it is often hard to point out symptoms, and up to a certain point with the disease, the individual may still appear cognitively normal [65]. Biomarkers enriched exosomes have been used in multiple studies for early detection and to follow the progression of these diseases because they are key indicators of specific changes

[66, 67, 68]. Exosomes derived from the blood allow a minimally invasive way of tracking therapeutic effects on disease progression [64]. Tracking exosomes in the blood has also been useful in tumor development and progression [69]. Exosomes identified in the blood can be collected and analyzed via liquid biopsy for tumor formation. This process is also much more feasible for large-scale implementation in comparison physical biopsies [70]. Exosomes carry biomarkers for tumors even in their early stages, allowing for earlier detection and treatment of cancer [71]. However, isolation and purification of exosomes from the blood sample remain challenging and are an obstacle that must be resolved for wide-scale clinical usage of this technique [71]. One study obtained results suggesting that purification was not needed to monitor cancer-derived EVs in circulation, providing a promising platform for monitoring cancer progression [72]. Blood derived exosomes open a variety of pathways in regenerative medicine and have been found to be beneficial in tracking disease progression. Future studies on blood-derived exosomes are required in relation to the detection and tracking of heart disease to advance this field further.

2.3. Embryonic Stem Cell Derived Exosomes

Embryonic stem cells (ESCs) have also shown great potential for cardiac tissue regeneration uses in the past. Their ability to differentiate to cardiomyocytes *in vitro* in high yields makes them advantageous over other stem cell therapies for the treatment of heart disease [73, 74]. Although ESCs are considered a more suitable donor cell source, making them more clinically translatable, tumor formation continues to be observed [75]. Additionally, ESCs suffer poor cell survival and functionality rates after transplantation [76]. Despite these setbacks revolving ESC therapies, in consolidation with new cell-free components, ESCs may still provide an important role in cardiac regenerative medicine.

ESC-derived exosomes offer similar benefits that regular ESCs provide, without some of the consequences of using cellular therapeutics. Both mouse ESCs and embryonic fibroblasts (EFs) exosomes have been shown to decrease infarct size when transplanted in mice post-MI [75]. Additionally, the ESC exosomes have been shown to increase capillary density and the number of cardiomyocytes found in the heart while it is recovering from ischemia [75]. ESCs are also thought to be largely responsible for increased CPC survival and proliferation both *in vitro* and *in vivo* [75]. ESCs provide an attractive cell-free alternative to treating myocardial injury and promoting tissue regeneration.

2.4. Biomimetic Nanoparticles

Aside from EVs, nanotechnology has gained interest in the past few years for treating heart disease. The development and functionalization of nanoparticles offer a promising platform for cardiac drug delivery. In recent studies, Su *et al.*, designed platelet inspired nanocells (PINCs), which showed the ability to continuously release pro-myogenic and pro-angiogenic paracrine factors. These PINCs were also found to promote the growth and proliferation of H9c2 cardiomyoblasts *in vitro* [19]. *In vivo*, mice with previous (I/R) injuries intravenously received PINCs [19]. After assessing the long-term effect of the nanocells on the recovery of the injured area, it was determined that various PINC treatments preserved and improved cardiac functions [19].

In a similar study to the design and development of PINCs, Liang *et al.*, used a unique nanoparticle platform for drug delivery purposes. In their study, they created an MSC and red blood cell (RBC) inspired nanoparticle, or MRIN [77]. This nanoparticle contains MSC secretomes in its core for the therapeutic aspect, and RBC membranes on the outer shell for further stabilization [77]. Although this study used their designed MRINs to target acute liver failure, this method could be used for the treatment of heart disease in the future. Liang *et al.*, created an advantageous drug delivery platform that passed common setbacks, such as hemocompatibility and future clinical translatability. The RBC component aids in hemocompatibility and gives MRIN a longer blood half-life [77]. Additionally, the usage of MSC secretomes versus regular MSCs allows easier shipping and long-term storage, making MRINs more readily available for clinical use [77].

Within nanomedicine, the targeted release and long-term retention of the therapeutic agent have been continuous issues with designed nanoparticles [18]. Enzyme-responsive nanoparticles have become a popular design within the field for targeted drug delivery post-MI to combat these problems. These nanoparticles incorporate nanomaterials with enzymatic responses to allow site-specific delivery of drugs [13]. The particles are designed to respond to an enzymatic trigger which then uniquely alters the physiochemical properties of the particle to release a drug [13]. One study by Nguyen *et al.*, used enzymatic stimuli responses, such as from matrix metalloproteinases (MMPs) to improve the specific targeting and delivery of the therapeutic release [18]. Using this strategy, nanoparticles are intravenously (IV) injected into the body and are able to target and treat the infarcted area based on the environmental levels of MMPs [18]. This method was shown to increase retention times of the responsive nanoparticles in the tissue and provided a minimally invasive strategy for delivering nanoparticles to the infarct area in an effort to treat MI in a rat model [18].

Another promising platform within nanomedicine is the ability to coat nanoparticles to combine the advantages of using natural and synthetic properties from nanomaterials. Researchers have utilized cellular membrane material to coat nanoparticles, allowing them to deliver tumor associated antigens for the treatment of cancer. The cellular membrane shell allows a more targeted drug delivery to the cancer and the synthesized nanoparticle core creates a more stabilized platform [78]. This nanoparticle coating approach provides a more natural solution towards the treatment of many diseases [78]. Although many studies revolving around these coated nanoparticles focus on the delivery of anticancer drugs, the platform provides a novel method for drug delivery to treat heart failure and promote cardiac tissue regeneration.

All of these unique biomimetic nanoparticle platforms provide an innovative pathway for cardiac regenerative medicine. Some of the approaches, such as PINCs, offer a platform that is continuously able to release treatment post-MI [19]. Other studies have worked on using different materials, such as cellular membrane, to use in various components of the nanoparticle such as the shell or the core [77, 78]. Coating the particle in various organic materials has aided in creating a more stabilized platform of drug delivery [78]. Enzyme-responsive nanoparticles have also become a novel approach to using natural stimuli found in the body to aid in drug delivery [18]. Although research within nanomedicine has

continued to grow, areas within the field such as pharmacokinetics, pharmacodynamics, and the safety of nanomaterials as a whole still challenge researchers [79]. Many studies have even emerged in looking towards platelet-based systems for drug delivery [79, 80, 81, 82]. These systems offer various advantages such as their long circulation lifespan and higher biocompatibility in comparison to nanoparticles [79]. In one study a system was even designed to pre-target platelets to engage with endogenous stem cells and then move to the infarcted area for cardiac repair [81]. In another study, platelet nanovesicles were fused to CSCs and were shown to have therapeutic benefits against ischemia in a rat model [82]. Nonetheless, there is still hope for biomimetic nanoparticles. As shown, they offer many advantageous benefits in cardiac regenerative medicine, and as future research continues, there is a possibility for their platform to grow and strengthen.

3. THE FUTURE FOR EVs IN REGENERATIVE MEDICINE

Clinical trials involving stem cell research have been around for decades, yet few research studies involving exosomes have moved towards clinical trials phases [83]. Many regulations and restrictions have been applied on how stem cell therapies can be used clinically. For example, homologous cell-based therapeutics do not require FDA approval, whereas manipulated autologous cell therapies require investigation and approval from the FDA before they can be used clinically [84].

While there are over 7,000 clinical trials registered related to stem cells, there are less than 200 registered trials on EVs and exosomes (www.clinicaltrials.gov). Since EVs are a more novel concept, the FDA guidelines on how to regulate clinical usage of them are still widely unclear [85]. As of now, the FDA considers EVs a biological medicine, thus EV therapeutics must follow FDA regulations under that category [85]. However, it is uncertain whether or not new rules specifically regulating the clinical usage of EVs will arise in the future to further standardize these procedures [85]. Although there are limitations for EVs currently, there is still a promising future for them in regenerative medicine.

3.1. Manufacturing and Commercialization Hurdles

In order to successfully complete the translation process of EVs into clinics, several issues regarding manufacturing and delivery methods must first be addressed.

Although EVs themselves offer a cell-free therapeutic platform, in order to obtain them, the culturing of cell systems is still required, which poses potential issues for clinical translatability. Minor changes in the culturing stages can render EVs' physical and chemical properties [85]. For example, the decision to use culture medium with or without fetal bovine serum (FBS) can lead to altered EV secretion [86]. Thus, a controlled procedure to manufacture EVs is needed for reproducibility. As mentioned earlier, there are currently several different methods for isolating and purifying exosomes. With non-uniform isolation methods comes the risk of contamination and differences in exosome properties and therapeutic abilities. Without a standardized isolation method, variability between exosome batches will remain an issue, one that must be overcome before thorough clinical integration of exosomal therapies [87].

In order for EVs to transition towards large-scale clinical applications, transportation and storage of EVs must also be considered. It has been found that −80°C is an optimal storage temperature for exosomes [87]. However, this can be somewhat complex when it comes to transportation of exosomes for clinical usage. Storing exosomes at higher temperatures has been tested, and it has been found that lyophilization may increase their stability when they are being stored or transported at temperatures above −80°C [87]. However, minimal testing has been done on this theory and the full effects of lyophilization and storing exosomes at an increased temperature are still unclear [87]. Additionally, other factors such as thawing procedures, pH shifts, and the container for transportation may affect the overall sample quality and thus their therapeutic efficacy [88]. All of these storage factors must be considered and standardized before EVs will be ready for large-scale clinical applications.

The ability to create an optimized and uniform protocol for isolating, purifying, storing, and transporting EVs is the key to commercialization. With that being said, creating those protocols that pass safety regulations and yield quality controlled EVs is quite tricky [88]. Another major obstacle in the way of being able to produce exosomes on a large-scale basis is their relatively low productivity. A useful dosage of exosomes is normally 10–100μg exosomal protein/mouse [89, 90]. Keeping in mind 1mL of culture medium typically produces not even 1μg of exosomes, it is easy to see how mass-producing exosomes for commercial uses can become complex and labor-intensive and expensive [87]. Although some studies have observed an increased yield of exosomes while culturing cells under conditions such as hypoxia or low pH, the extent to which these environments have an impact on the composition of exosomal components is unclear [87]. Once standardization of protocols occurs, it is likely that clinical trials for EVs will be able to progress.

3.2. Delivery and Dosage

Optimizing delivery and dosage methods of EV therapeutics is another aspect that must be considered before their advancement into the clinical world. Different delivery methods, such as injections via various routes, have been explored [91]. One study used cardiac homing peptides (CHPs) for a more targeted approach of delivering exosomes intravenously [92]. It was found that exosomes labelled with CHPs decreased infarcted tissue in a rat model [92]. Another study demonstrated the effectiveness of intravenously delivered CSCderived exosomes for the treatment of dilated cardiomyopathy [93]. How- ever, researchers are still in the process of identifying which method works best for corresponding procedures. Since exosomes are taken up by macrophages, it can be tricky to control their biodistribution and determine where majority of them will accumulate [87]. Currently, an assortment of methods for determining exosome dosage exists. Techniques such as flow cytometry, nanoparticle tracking analysis (NTA), and tunable resistive pulse sensing (TRPS) to name a few, allow verification of particle size and the concentration of a sample [90]. However, without standardized procedures for the preparation of exosomes, quantifying dosages be- come complex and may vary from case to case. Even for cell-based therapies, there are still struggles with determining the best dosing amounts. One study demonstrated the dose-dependent benefits of intramyocardial injection of human cardiospheres for the treatment of acute MI in a mouse model [94]. However, there are still more studies to be done before a definite dosing range can be confirmed for these types of therapies. Potency

assays are rising as great tools for dosing exosomes by quantifying each exosome batch's bioactive load. They have the potential to decrease dosing inconsistencies between batches and overall help exosome therapies to proceed toward clinical uses [90]. Determining the best cell source for exosomal therapies is another variable researchers must continue to study. As shown, there have been numerous studies involving exosomes derived from different stem cell types, as well as exosomes derived from blood. One study even narrowed down their exosome source between cardiac stromal cells from patients with heart failure and healthy patients [95]. In this study it was shown that the exosomes derived from patients with heart failure impaired therapeutic potency post MI in a mouse model [95]. This shows the many factors to consider when choosing an exosome source and how various details, such as the health condition of the exosome source, can alter the overall efficiency of the treatment. Optimization of delivery and dosage of EVs in the future can help progress more clinical trials and accelerate translation for clinical usage.

3.3. Moving Forward

In addition to standardization of protocols, more large animal studies involving EV therapies are needed before clinical translation and commercialization. Currently, human EV models present low species specificities, meaning these therapies should translate fairly well from animal studies towards humans [85]. However, additional animal models of EV therapeutics help researchers better understand possible toxicities and specific effects of certain platforms [85]. Thus, increased animal studies can aid in preclinical research that may help speed up the process of translating EVs to clinics for humans [85]. Current results of Phase I clinical studies have proven EV therapeutics to be a feasible and safe option for treating various diseases [85]. However, assessing long term effects of these therapies will be crucial in helping other EV trials get approved [85].

CONCLUSION

Exosome therapeutics and biomimetic nanoparticle platforms have the potential to provide unique approaches for treating various diseases, including heart disease. Their cell-free aspect sets encouraging expectations to bypass common issues with other cellular based therapies. Increasing research on various types of exosomes and their benefits aids in providing a better understanding of their novel concept and their many different applications. Additionally, biomimetic nanoparticles to treat various diseases have gained therapeutic potential within nanomedicine. These nanoparticles offer a great way to integrate biological and synthetic components to create advantageous platforms for regenerative medicine. The increasing amount of research being done on both EVs and biomimetic nanoparticles is promising for their translation towards clinical usage. However, standardized protocols for many aspects of exosome therapeutics are needed before they will be able to scale-up and become widely commercialized. More consistent results across different animal studies for various diseases are also needed to move forward clinically. Many studies mentioned throughout this review are still in early stages of research, and the topic of EVs and nanomedicine as a whole is still a fairly modern concept to the world of regenerative medicine. Nonetheless, the results gained from these studies play a crucial factor in the research process and provide valuable knowledge for future researchers to

expand upon. However, the reliability of the studies mentioned may not always hold true as new research and findings in this topic come forward in the future. Additionally, none of the studies discussed were on human models, therefore translating them over to human models may be more complex than anticipated. Exosomes, and nanomedicine as a whole are growing topics in cardiac regenerative medicine, they have the capability to greatly advance regenerative medicine as research on these topics continues and current challenges are being overcome.

FUNDING

This study was supported by the U.S. National Institute of Health (HL123920, HL137093, HL144002, HL146153, and HL147357) and American Heart Association (18TPA34230092 and 19EIA34660286) to K.C.

LIST OF ABBREVIATIONS

EVs	Extracellular vesicles
I/R	Ischemia/reperfusion
MI	Myocardial infarction
MVBs	Multivesicular bodies
PTX	Paclitaxel
BBB	Blood brain barrier
SEC	Size exclusion chromatography
MVs	Microvesicles
HUVEC	Human umbilical vein endothelial cell
CPC	Cardiac progenitor cell
iPSC	Induced pluripotent stem cell
MPC	Multipotential progenitor cell
MSC	Mesenchymal stem cell
CSC	Cardiac stem cell
C-MSC	Cardiac mesenchymal stem cell
UCB	Umbilical cord blood
IUGR	Intrauterine growth restriction
ESC	Embryonic stem cell
RBC	Red blood cell
EF	Embryonic fibroblast

MMP	Matrix metalloproteinase
IV	Intravenous
FBS	Fetal bovine serum
CHP	Cardiac homing peptide
NTA	Nanoparticle tracking analysis
TRPS	Tunable resistive pulse sensing

REFERENCES

[1]. Heron M Deaths: Leading Causes for 2016. Natl Vital Stat Rep 2018; 67(6): 1–77.https://www.cdc.gov/nchs/data/nvsr/nvsr67/nvsr67_06.pdf

[2]. Roth GA, Johnson CO, Abate KH, et al. The burden of cardiovascular dis- eases among us states, 1990–2016. JAMA Cardiol 2018; 3(5): 375–89. [PubMed: 29641820]

[3]. Li Z, Hu S, Cheng K. Chemical Engineering of Cell Therapy for Heart Diseases. Acc Chem Res 2019; 52(6): 1687–96. [PubMed: 31125198]

[4]. Amariglio N, Hirshberg A, Scheithauer B, et al. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient PLoS Medicine 2009; 6(2): 0221–31.http://dx.plos.org/10.1371/journal.pmed.1000029

[5]. Erdö F, Bührle C, Blunk J, et al. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke Blood Flow Metab 2003; 23(7): 780–5.https://journals.sagepub.com/doi/full/10.1097/01.WCB.0000071886

[6]. Herberts C, Kwa M, Hermsen H. Risk factors in the development of stem cell therapy. Journal of Translational Medicine 2011; 9(29): 1–14.medicine.biomedcentral.com/articles/10.1186/1479-5876-9-29 10.1186/1479-5876-9-29

[7]. Tang J, Cores J, Huang K, et al. Concise Review: Is Cardiac Cell Therapy Dead? Embarrassing Trial Outcomes and New Directions for the Future Stem Cells TM 2018; 7(4): 354–9.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5866934/pdf/SCT3-7-354.pdf

[8]. Heslop JA, Hammond TG, Santeramo I, et al. Concise review: work-shop review: understanding and assessing the risks of stem cell-based therapies. Stem Cells Transl Med 2015; 4(4): 389–400. [PubMed: 25722427]

[9]. Chistiakov DA, Orekhov AN, Bobryshev YV. Cardiac extracellular vesi- cles in normal and infarcted heart. Int J Mol Sci 2016; 17(1): 63

[10]. Zha Q, Yao Y, Ren Z, Li X, Tang J. Extracellular vesicles: An overview of biogenesis, function, and role in breast cancer Tumor Biology 2016; 39(2)http://journals.sagepub.com/doi/full/10.1177/1010428317691182?url_ver=Z39.88-

[11]. Jargin S Orekhov's Method: Reassessment of In vitro Lipid Uptake Assays Recent Advances in Cardiovascular Drug Discorvery 2014; 9(2): 122–5.http://www.eurekaselect.com/132845/article

[12]. Balmert SC, Little SR. Biomimetic delivery with micro- and nanoparticles. Adv Mater 2012; 24(28): 3757–78. [PubMed: 22528985]

[13]. Hua Q, Kattic P, Gu Z. Enzyme-Responsive Nanomaterials for Controlled Drug Delivery. Nanoscale 2014; 176(21): 139–48.

[14]. Jin K, Luo Z, Zhang B, Pang Z. Biomimetic nanoparticles for inflammation targeting. Acta Pharm Sin B 2018; 8(1): 23–33. [PubMed: 29872620]

[15]. Dvir T, Bauer M, Schroeder A, et al. Nanoparticles targeting the infarcted heart. Nano Lett 2011; 11(10): 4411–4. [PubMed: 21899318]

[16]. Emilienne C, Dubernet C, Bentolila D, Benita S, Couvreur P. Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles Biomaterials 2000; 21(1): 1–7.https://www.sciencedirect.com/science/article/pii/S01429612990012 [PubMed: 10619673]

[17]. Kennedy LC, Bickford LR, Lewinski NA, et al. A new era for cancer treatment: gold-nanoparticle-mediated thermal therapies. Small 2011; 7(2): 169–83. [PubMed: 21213377]

[18]. Nguyen MM, Carlini AS, Chien MP, et al. Enzyme-Responsive Nanoparticles for Targeted Accumulation and Prolonged Retention in Heart Tissue after Myocardial Infarction. Adv Mater 2015; 27(37): 5547–52. [PubMed: 26305446]

[19]. Su T, Huang K, Ma H, et al. Platelet-Inspired Nanocells for Targeted Heart Repair After Ischemia/Reperfusion Injury. Adv Funct Mater 2019; 29(4): 4–14.

[20]. Sun D, Zhuang X, Zhang S, et al. Exosomes are endogenous nanoparticles that can deliver biological information between cells Advanced Drug Delivery Reviews 2012; 65(3): 342–7.https://www.sciencedirect.com/science/article/pii/S0169409X12002 [PubMed: 22776312]

[21]. Bellin G, Gardin C, Ferroni L, et al. Exosome in Cardiovascular Diseases: A Complex World Full of Hope. Cells 2019; 8(2): 166

[22]. Lai R, Yeo R, Tan K, Lim S. Exosomes for drug delivery - A novel application for the mesenchymal stem cell Biotechnology Advances 2013; 31(5): 543–1. https://www.sciencedirect.com/science/article/pii/S0734975012001 [PubMed: 22959595]

[23]. Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, Sánchez-Madrid F, Mittelbrunn M. Sorting it out: regulation of exosome loading. Semin Cancer Biol 2014; 28: 3–13. [PubMed: 24769058]

[24]. Sinha S, Hoshino D, Hong N, et al. Cortactin promotes exosome secretion by controlling branched actin dynamics JCB 2016; 214(2): 197–213.http://jcb.rupress.org/cgi/pmidlookup?view=long&pmid=2740295 [PubMed: 27402952]

[25]. Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. Trends Cell Biol 2017; 27(3): 172–88. [PubMed: 27979573]

[26]. Kanemoto S, Nitani R, Murakami T, et al. Multivesicular body formation enhancement and exosome release during endoplasmic reticulum stress Biochemical and Biophysical Research Communications 2016; 480(2): 166–72.https://www.sciencedirect.com/science/article/pii/S0006291X1631 [PubMed: 27725157]

[27]. King H, Michael M, Gleadle J. Hypoxic enhancement of exosome release by breast cancer cells BMC Cancer 2012; 12(421)

[28]. Vader P, Mol E, Pasterkamp G, Schiffelers R. Extracellular vesicles for drug delivery Extracellular vesicles for drug delivery Advanced Drug Delivery Reviews 2016; 106: 148–56. [PubMed: 26928656]

[29]. Pascucci L, Coccè V, Bonomi A, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: A new approach for drug delivery Journal of Controlled Release 2014; 192: 262–70.https://www.sciencedirect.com/science/article/pii/S0168365914005 [PubMed: 25084218]

[30]. Haney MJ, Klyachko NL, Zhao Y, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release 2015; 207: 18–30. [PubMed: 25836593]

[31]. Yang T, Martin P, Fogarty B, et al. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharm Res 2015; 32(6): 2003–14. [PubMed: 25609010]

[32]. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakahl S, Woos M. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 2011; 29(4): 306–9. 10.1038/nbt.1807 [PubMed: 21614804]

[33]. Morales-Prieto D, Stojiljkovic M, Diezel C, et al. Peripheral blood exosomes pass blood-brain-barrier and induce glial cell activation bioRxiv 2019https://www.biorxiv.org/content/10.1101/471409v1.full

[34]. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. Theranostics 2017; 7(3): 789–804. [PubMed: 28255367]

[35]. Yu L, Zhu J, Liu J, et al. A Comparison of Traditional and Novel Methods for the Separation of Exosomes from Human Samples Bio-Med Research International 2018; 2018

[36]. Gardiner C, Di Vizio D, Sahoo S, et al. Techniques used for the isolation and characterization of extracellular vesicles: results of a world-wide survey. J Extracell Vesicles 2016; 5(1): 32945 [PubMed: 27802845]

[37]. Rood IM, Deegens JK, Merchant ML, et al. Comparison of three methods for isolation of urinary microvesicles to identify biomarkers of nephrotic syndrome. Kidney Int 2010; 78(8): 810–6. 10.1038/ki.2010.262 [PubMed: 20686450]

[38]. Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. Proteomics 2008; 8(19): 4083–99. [PubMed: 18780348]

[39]. Chen Y, Li G, Liu ML. Microvesicles as Emerging Biomarkers and Therapeutic Targets in Cardiometabolic Diseases. Genomics Proteomics Bioinformatics 2018; 16(1): 50–62. [PubMed: 29462670]

[40]. Menck K, Sönmezer C, Worst TS, et al. Neutral sphingomyelinases control extracellular vesicles budding from the plasma membrane. J Extracell Vesicles 2017; 6(1)1378056https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5699186 / http://dx.doi.org/10.1080/20013078.2017.1378056http://dx.doi.org/10.1080/20013078.2017.1378056http://dx.doi.org/10.1080/20013078.2017.1378056 [PubMed: 29184623]

[41]. Wang N, Chen C, Yang D, et al. Mesenchymal stem cells-derived extracellular vesicles, via miR-210, improve infarcted cardiac function by promotion of angiogenesis Biochimica et Biophysica Acta 1863; 1863(8): 2085–92.https://www.sciencedirect.com/science/article/pii/S0925443917300

[42]. Wang Y, Chen LM, Liu ML. Microvesicles and diabetic complications--novel mediators, potential biomarkers and therapeutic targets. Acta Pharmacol Sin 2014; 35(4): 433–43. 10.1038/aps.2013.188 [PubMed: 24608676]

[43]. Bollini S, Smart N, Riley P. Resident cardiac progenitor cells: At the heart of regeneration Journal of Molecular and Cellular Cardiology 2011; 50(2): 296–303.https://www.sciencedirect.com/science/article/pii/S002228281000 [PubMed: 20643135]

[44]. Silvestre JS. Pro-angiogenic cell-based therapy for the treatment of ischemic cardiovascular diseases. Thromb Res 2012; 130(Suppl. 1): S90–4. 10.1016/j.thromres.201208.287 [PubMed: 23026676]

[45]. Amini H, Rezaie J, Vosoughi A, Rahbarghazi R, Nouri M. Cardiac progenitor cells application in cardiovascular disease. J Cardiovasc Thorac Res 2017; 9(3): 127–32. [PubMed: 29118944]

[46]. Davis DR, Zhang Y, Smith RR, et al. Validation of the cardiosphere method to culture cardiac progenitor cells from myocardial tissue. PLoS One 2009; 4(9)e7195 [PubMed: 19779618]

[47]. Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002; 106(24): 3009–17. [PubMed: 12473544]

[48]. Lu TY, Lin B, Kim J, et al. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nat Commun 2013; 4(2307): 2307 [PubMed: 23942048]

[49]. Guo J, Zheng D, Li WF, Li HR, Zhang AD, Li ZC. Insulin-like growth factor 1 treatment of MSCs attenuates inflammation and cardiac dysfunction following MI. Inflammation 2014; 37(6): 2156–63. [PubMed: 24942913]

[50]. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. Mol Ther 2015; 23(5): 812–23. [PubMed: 25868399]

[51]. Xiang MX, He AN, Wang JA, Gui C. Protective paracrine effect of mesenchymal stem cells on cardiomyocytes. J Zhejiang Univ Sci B 2009; 10(8): 619–24. [PubMed: 19650201]

[52]. Huang K, Hu S, Cheng K. A New Era of Cardiac Cell Therapy: Opportunities and Challenges. Adv Healthc Mater 2019; 8(2)e1801011https://onlinelibrary.wiley.com/doi/pdf/10.1002/adhm.201801011 [PubMed: 30548836]

[53]. Tang J, Wang J, Huang K, et al. Cardiac cell-integrated microneedle patch for treating myocardial infarction. Sci Adv 2018; 4(11)eaat9365 [PubMed: 30498778]

[54]. Su T, Huang K, Daniele MA, et al. Cardiac Stem Cell Patch Integrated with Microengineered Blood Vessels Promotes Cardiomyocyte Proliferation and Neovascularization after Acute Myocardial Infarction. ACS Appl Mater Interfaces 2018; 10(39): 33088–96. [PubMed: 30188113]

[55]. Tang J, Cui X, Caranasos TG, et al. Heart Repair Using Nanogel-Encapsulated Human Cardiac Stem Cells in Mice and Pigs with Myocardial Infarction. ACS Nano 2017; 11(10): 9738–49. [PubMed: 28929735]

[56]. Cui X, Tang J, Hartanto Y, et al. NIPAM-based Microgel Microenvironment Regulates the Therapeutic Function of Cardiac Stromal Cells. ACS Appl Mater Interfaces 2018; 10(44): 37783–96. [PubMed: 30360109]

[57]. Cores J, Caranasos TG, Cheng K. Magnetically Targeted Stem Cell Delivery for Regenerative Medicine. J Funct Biomater 2015; 6(3): 526–46. [PubMed: 26133387]

[58]. Teng X, Chen L, Chen W, Yang J, Yang Z, Shen Z. Mesenchymal stem cell-derived exosomes improve the microenvironment of infarcted myocardium contributing to angiogenesis and anti-inflammation. Cell Physiol Biochem 2015; 37(6): 2415–24. [PubMed: 26646808]

[59]. Huang P, Wang L, Li Q, et al. Atorvastatin enhances the therapeutic efficacy of mesenchymal stem cells-derived exosomes in acute myocardial infarction via up-regulating long non-coding RNA H19. Cardiovasc Res 2020; 116(2): 353–67.https://www.ncbi.nlm.nih.gov/pubmed/31119268 [PubMed: 31119268]

[60]. Ju C, Shen Y, Ma G, et al. Transplantation of Cardiac Mesenchymal Stem Cell-Derived Exosomes Promotes Repair in Ischemic Myocardium. J Cardiovasc Transl Res 2018; 11(5): 420–8. [PubMed: 30232729]

[61]. He B, Li X, Yu H, Zhou Z. Therapeutic potential of umbilical cord blood cells for type 1 diabetes mellitus. J Diabetes 2015; 7(6): 762–73. 10.1111/1753-0407.12286 [PubMed: 25799887]

[62]. Hu Y, Rao SS, Wang ZX, et al. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21–3p-mediated promotion of angiogenesis and fibroblast function. Theranostics 2018; 8(1): 169–84. [PubMed: 29290800]

[63]. Luo J, Fan Y, Shen L, et al. The pro-angiogenesis of exosomes derived from umbilical cord blood of intrauterine growth restriction pigs was repressed associated with MiRNAs. Int J Biol Sci 2018; 14(11): 1426–36. [PubMed: 30262994]

[64]. Abdel-Haq H Blood exosomes as a tool for monitoring treatment efficacy and progression of neurodegenerative diseases. Neural Regen Res 2019; 14(1): 72–4. [PubMed: 30531076]

[65]. Mustapic M, Eitan E, Werner J, et al. Plasma extracellular vesicles enriched for neuronal origin: A potential window into brain pathologic processes 2017

[66]. Athauda D, Gulyani S, Karnati H, et al. Utility of Neuronal-Derived Exosomes to Examine Molecular Mechanisms That Affect Motor Function in Patients With Parkinson Disease JAMA Neurology 2019; 76(4): 420–9. [PubMed: 30640362]

[67]. Jack CR Jr, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010; 9(1): 119–28. 10.1016/S1474-4422(09)70299-6 [PubMed: 20083042]

[68]. Wroblewska L, Kitada T, Endo K, et al. Identification of pre-clinical Alzheimer's disease by a profile of pathogenic proteins in neurally-derived blood exosomes: a case-control study. Alzheimers Dement 2015; 33(8): 839–41.https://dx.doi.org/10.1016%2Fj.jalz.2014.06.008

[69]. Zhang W, Xia W, Lv Z, Ni C, Xin Y, Yang L. Liquid Biopsy for Cancer: Circulating Tumor Cells, Circulating Free DNA or Exosomes? Cell Physiol Biochem 2017; 41(2): 755–68. 10.1159/000458736 [PubMed: 28214887]

[70]. Contreras-Naranjo JC, Wu HJ, Ugaz VM. Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine. Lab Chip 2017; 17(21): 3558–77. [PubMed: 28832692]

[71]. He M, Zeng Y. Microfluidic Exosome Analysis toward Liquid Biopsy for Cancer. J Lab Autom 2016; 21(4): 599–608. [PubMed: 27215792]

[72]. Yoshioka Y, Kosaka N, Konishi Y, et al. Ultra-sensitive liquid biopsy of circulating extracellular vesicles using ExoScreen. Nat Commun 2014; 5(3591): 3591 [PubMed: 24710016]

[73]. Helgason C, Miller C, Dang S, Zandstra P. Scalable Production of Embryonic Stem Cell-Derived Cells. Tissue Engineering 2004; 9(4): 353–64.https://www.liebertpub.com/doi/full/10.1089/107632703768247

[74]. Hodgson DM, Behfar A, Zingman LV, et al. Stable benefit of embryonic stem cell therapy in myocardial infarction. Am J Physiol Heart Circ Physiol 2004; 287(2): H471–9. [PubMed: 15277190]

[75]. Khan M, Nickoloff E, Abramova T, et al. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. Circ Res 2015; 117(1): 52–64. [PubMed: 25904597]

[76]. Li J, Christophersen N, Hall V, Soulet D, Brundin P. Critical issues of clinical human embryonic stem cell therapy for brain repair. Trends in Neurosciences 2008; 31(3): 146–53. [PubMed: 18255164]

[77]. Liang H, Huang K, Su T, et al. Mesenchymal Stem Cell/Red Blood Cell-Inspired Nanoparticle Therapy in Mice with Carbon Tetrachloride-Induced Acute Liver Failure. ACS Nano 2018; 12(7): 6536–44. [PubMed: 29943967]

[78]. Fang RH, Hu CM, Luk BT, et al. Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. Nano Lett 2014; 14(4): 2181–8. [PubMed: 24673373]

[79]. Li Z, Hu S, Cheng K. Platelets and their biomimetics for regenerative medicine and cancer therapies. J Mater Chem B Mater Biol Med 2018; 6(45): 7354–65. [PubMed: 31372220]

[80]. Shen D, Li Z, Hu S, et al. Antibody-Armed Platelets for the Regenerative Targeting of Endogenous Stem Cells. Nano Lett 2019; 19(3): 1883–91. [PubMed: 30775924]

[81]. Li Z, Shen D, Hu S, et al. Pretargeting and Bioorthogonal Click Chemistry-Mediated Endogenous Stem Cell Homing for Heart Repair. ACS Nano 2018; 12(12): 12193–200. [PubMed: 30511851]

[82]. Tang J, Su T, Huang K, et al. Targeted repair of heart injury by stem cells fused with platelet nanovesicles. Nat Biomed Eng 2018; 2: 17–26. [PubMed: 29862136]

[83]. Halme DG, Kessler DA. FDA regulation of stem-cell-based therapies. N Engl J Med 2006; 355(16): 1730–5.https://www.researchgate.net/publication/6744705_FDA_Regulation [_of_Stem-Cell-Based_Therapies]. 10.1056/NEJMhpr063086 [PubMed: 17050899]

[84]. George B. Regulations and guidelines governing stem cell based products: Clinical considerations. Perspect Clin Res 2011; 2(3): 94–9. [PubMed: 21897884]

[85]. Lener T, Gimona M, Aigner L, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 2015; 4(1): 30087 [PubMed: 26725829]

[86]. Ramirez MI, Amorim MG, Gadelha C, et al. Technical challenges of working with extracellular vesicles. Nanoscale 2018; 10(3): 881–906. [PubMed: 29265147]

[87]. Yamashita T, Takahashi Y, Takakura Y. Possibility of Exosome-Based Therapeutics and Challenges in Production of Exosomes Eligible for Therapeutic Application. Biol Pharm Bull 2018; 41(6): 835–42. [PubMed: 29863072]

[88]. Fuster-Matanzo A, Gessler F, Leonardi T, Iraci N, Pluchino S. Acellular approaches for regenerative medicine: on the verge of clinical trials with extracellular membrane vesicles? Stem Cell Res Ther 2015; 6(1): 227 [PubMed: 26631254]

[89]. Lv LL, Wu WJ, Feng Y, Li ZL, Tang TT, Liu BC. Therapeutic application of extracellular vesicles in kidney disease: promises and challenges. J Cell Mol Med 2018; 22(2): 728–37.https://dx.doi.org/10.1111%2Fjcmm.13407 [PubMed: 29083099]

[90]. Willis GR, Kourembanas S, Mitsialis SA. Toward Exosome-Based Therapeutics: Isolation, Heterogeneity, and Fit-for-Purpose Potency. Front Cardiovasc Med 2017; 4: 63 [PubMed: 29062835]

[91]. Wiklander OP, Nordin JZ, O'Loughlin A, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. J Extracell Vesicles 2015; 4(26316): 26316 10.3402/jev.v4.26316 [PubMed: 25899407]

[92]. Vandergriff A, Huang K, Shen D, et al. Targeting regenerative exosomes to myocardial infarction using cardiac homing peptide. Theranostics 2018; 8(7): 1869–78. [PubMed: 29556361]

[93]. Vandergriff A, Andrade J, Tang J, et al. Intravenous Cardiac Stem Cell-Derived Exosomes Ameliorate Cardiac Dysfunction in Doxorubicin Induced Dilated Cardiomyopathy Stem Cells International 2015; 2015

[94]. Shen D, Cheng K, Marbán E. Dose-dependent functional benefit of human cardiosphere transplantation in mice with acute myocardial infarction. J Cell Mol Med 2012; 16(9): 2112–6. [PubMed: 22225626]

[95]. Qiao L, Hu S, Liu S, et al. microRNA-21–5p dysregulation in exosomes derived from heart failure patients impairs regenerative potential. J Clin Invest 2019; 129(6): 2237–50. [PubMed: 31033484]

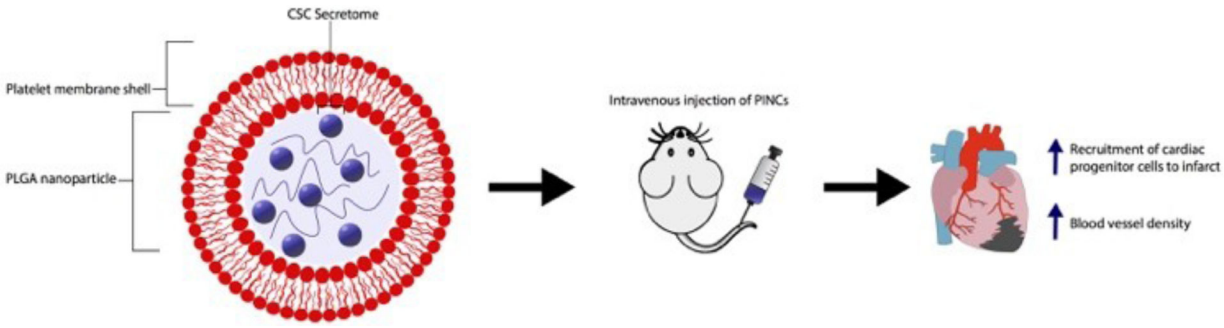

Fig. (1).
Intravenous injection of platelet inspired nanoparticles improves cardiac functions.