SCOPING REVIEW ON AVAILABLE DELIVERY SYSTEMS OF SHORT INTERFERING RNA (SIRNA) THERAPY TO TREAT CARDIOVASCULAR DAMAGE DURING COVID-19: A STUDY ON HUMAN AND ANIMAL MODELS

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ABSTRACT

Background: Short-interfering RNA (siRNA) has the potential to treat a variety of diseases by silencing specific genes. It is currently being considered as an alternative method to treat cardiovascular damage that developed after the Covid-19 infection. If the damage persists for an extended period of time, it will lead to several other cardiovascular problems such as myocarditis. Application of compatible delivery systems can overcome the immunogenicity caused by the off-target reactions and sensitive interaction of siRNA with other cells. Therefore, the purpose of this scoping review was to identify effective delivery systems of siRNA therapy for treating arising cardiovascular problems during Covid-19 based on current studies. **Methods:** The search applied appropriate keywords involving three online databases: PubMed, Scopus, and ScienceDirect. The article selection was based on Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guideline and all articles published between 2010 and 2022 that met the inclusion and exclusion criteria were considered. **Results:** A total of six articles were discovered. Only one study used human cells, while the other five studies used animal models to investigate the effectiveness of distinct types of siRNA in silencing gene expression related to specific cardiovascular damage, which are ischemia-reperfusion injury, myocarditis, myofibroblast, venous thrombosis, and atherosclerosis. The methods of siRNA delivery involved the application of naked siRNA, specific-targeting nanocomplexes, and extracellular vesicles, which were administered through chemical transfection, intravenous injection, and subcutaneous injection. **Conclusion:** Both *in vivo* and *in vitro* approaches have their respective compatible delivery methods and administration routes for each type of siRNA.

KEYWORDS: delivery system, Covid-19, short interfering RNA, siRNA, cardiovascular

INTRODUCTION

Covid-19 has impacted various organs, especially the sites with angiotensin-converting enzyme 2 (ACE2) receptors as the binding site or the cellular pathway for the SARS-CoV-2, such as the heart, lungs, kidney, liver and brain. According to Zaim et al. (2020), Covid-19 infection has different paces of fatality between body systems. The respiratory system was affected in 17% of patients, with 65% experiencing rapid deterioration. Other than that, the loss of renal function might be observed one week after infection. Meanwhile, the prolonged silent damage to the cardiovascular system has led to a number of non-survivors, including 24% with coronary heart disease, 48% with hypertension, and cardiovascular risk factors of 31.4% with diabetes mellitus and 9.0% due to smoking. Overall, most body systems exhibit symptoms within a few days following the infection except for the cardiovascular system. Many Covid-19 patients are exposed to the risk of long-covid damage asymptomatically. The term "long-covid" refers to the damage that develops post-infection of Covid-19, which may take up to weeks until months. All patients who received one dose of the vaccination, received all of the vaccine

doses, and previously tested positive for SARS-CoV-2 had an increased risk of myocarditis (Patone et al., 2021). Meanwhile, patients may have a reduced risk of pericarditis after vaccination but an increased risk if they are past positive patients. Lastly, the risk of developing cardiac arrhythmia only decreased upon the first dose, but it increased after the second dose or had a previous Covid-19 history. From the data, it can be inferred that vaccinated people still have the risk of developing cardiovascular problems.

Recently, gene silencing RNA or known as short-interfering (siRNA) therapy has been explored as a potential alternative to overcome the problem. However, it is exposed to the possibility of immunogenic reactions and toxicity during the process (Medeiros et al., 2021). Hence, a thorough analysis of the delivery systems is crucial to prevent toxicity between the biomaterials used, siRNA genes and the target sites or its surrounding cells. Several studies were conducted to examine the impact of various delivery systems for siRNA therapy. However, there is still a limited understanding of which delivery systems are suitable and available for treating Covid-19 using the siRNA.

This scoping review aims to provide an evidence-based analysis of the available studies on delivery systems that can be used to treat cardiovascular problems and reduce complications during COVID-19. The present review summarises effective delivery system used for cardiovascular damage, comprised of the type of siRNA used for silencing specific gene, as well as suitable method of delivery and its route of administration.

MATERIALS AND METHODS

Study Design

A scoping review was performed by gathering evidences based on the published research articles on the same topic of interest; delivery systems used to treat cardiovascular damages. The framework involved the construction of research objectives based on the research problems, selection of articles that met the goals of this review, screening process to ensure the conformation of inclusion and exclusion criteria and the availability of full-text articles.

Search Strategy

The literatures were chosen from three online databases that were reliable in providing data relevant to the medical and health research, which were PubMed, Scopus, and ScienceDirect. The database search included papers from 2010 to 2022 comprising of the combination of following keywords; 'delivery system', 'vehicle', 'biomaterial', 'transport', 'biomaterial', 'short-interfering RNA', 'siRNA', 'RNA interference', 'RNAi', 'Covid-19', 'Coronavirus 2019', 'SARS-CoV-2', 'cardiovascular', 'heart' and 'cardiac'. In addition, Boolean operators AND and OR were applied to assist in the finding of specific articles related to the study.

Inclusion and Exclusion Criteria

Several criteria were devised and considered during the selection of relevant papers for this scoping review. Articles that did not meet the inclusion criteria outlined in Table 1 were excluded from the study.

Table 1 Inclusion and exclusion criteria for article selection

Inclusion Criteria	Exclusion Criteria
a) Published research articles were written in English. b) All articles must be published between 2010 and 2022. c) Qualitative and quantitative studies. d) Papers related to delivery systems of short interfering RNA (siRNA) and cardiovascular damage. e) The search consists of specific keywords.	a) Publish research articles were written in non-English version. b) Articles were published before 2010. c) Chapter of books, reviewed articles, proceeding, conference presentation, dissertation, and discussion papers. d) Absence of abstract and full-text articles e) Papers were not relevant to the objectives of the study.

Selection Procedures

The selection of articles was based on Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guideline (Moher et al., 2009). Articles published between 2010 and 2022 were extracted from online databases and screened. After removing duplicates from multiple databases, the selection of articles was subjected to title and abstract screening to identify any irrelevant studies. Then, all full-text articles of the remaining papers were retrieved. Following that, the information in those articles were examined thoroughly to analyse the data on the delivery systems of siRNA therapy in treating cardiovascular problems in Covid-19 patients. Throughout the screening process, articles that violated inclusion criteria and conformed with exclusion criteria will be excluded. The selection procedures are displayed in the PRISMA flow diagram shown in Figure 1.

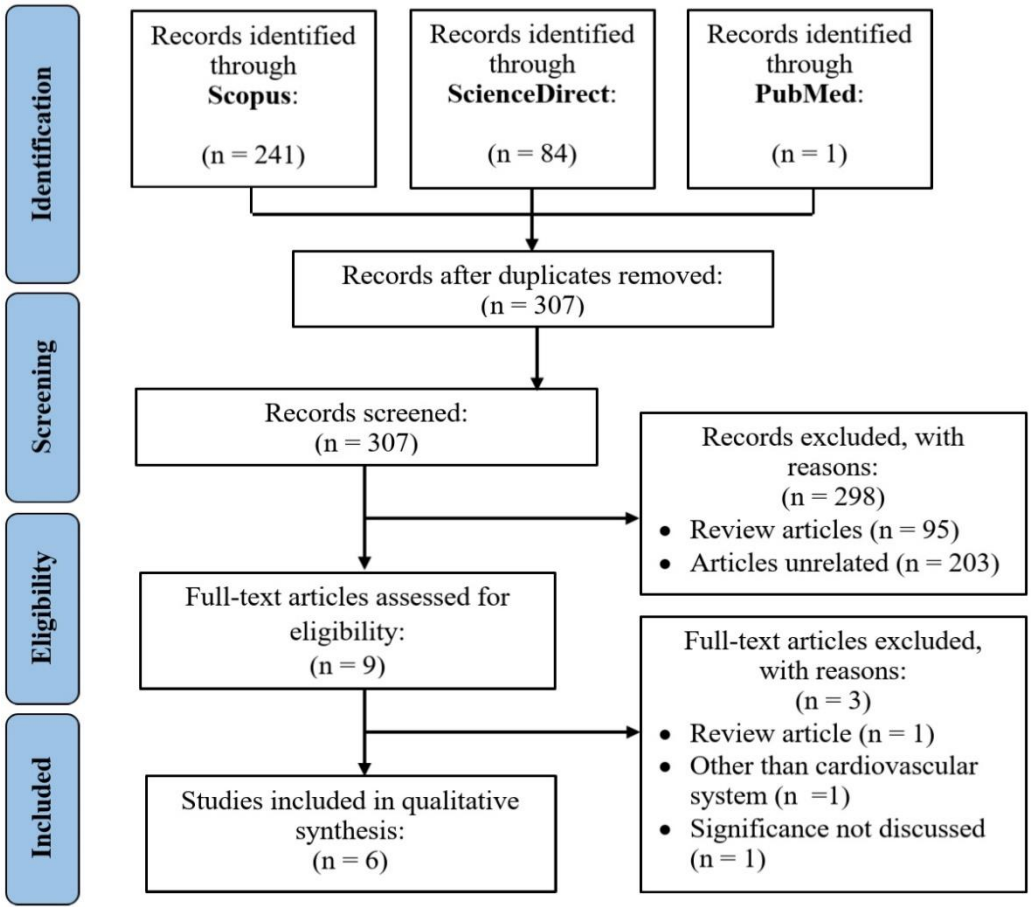

Figure 1 PRISMA flow diagram (Moher et al., 2009)

Data Extraction

The findings from the final full-text articles that passed the screening processes for the scoping review were extracted, analysed, and presented using tables. The tables have included important information about final full-text articles comprising the author's name, year of publication, method of study and subjects or models involved in the studies. The main findings of this scoping review are the characteristics of the delivery systems used in siRNA therapy in treating cardiovascular problems among Covid-19 patients, which comprised of the types of cardiovascular damage, type of short-interfering RNA, methods of delivery, routes of administration and impacts of the delivery system used.

RESULTS AND DISCUSSIONS

The selection of articles is summarised in the Figure 1. A total of 326 articles were discovered through Scopus, ScienceDirect and PubMed databases. After removing 19 duplicates, 307 remaining articles were assessed for the title and abstract screening. A total of 298 irrelevant articles were eliminated including 95 review articles and 203 articles unrelated to siRNA delivery in treating cardiovascular damage. To evaluate compliance with the inclusion and exclusion criteria, the full texts of the remaining publications were retrieved and reviewed. Three papers that met the exclusion criteria were excluded,

including a review and an article on the use of siRNA in the respiratory system. Finally, only six articles remained and specifically focused on the siRNA delivery for cardiovascular problems. The findings from the final full-text articles were synthesised and tabulated in Table 2.

Both *in vitro* and *in vivo* approaches used the same type of siRNA and method of delivery but different study model and route of administration for each cardiovascular damage. As shown Table 2, each cardiovascular damage utilised 6 distinct types of siRNA. Among the 6 final articles, 2 articles used each method of siRNA delivery, consisting of naked siRNA, specific-targeting nanocomplexes and extracellular vesicles. In all *in vitro* studies, chemical transfection was used as the route of administration, while intravenous and subcutaneous injections were used in *in vivo* studies. Table 3 summarised the effectiveness of each delivery system in all six final articles through positive recovery or changes observed in respective cardiovascular damage tested on.

The application of siRNA to treat cardiovascular problems of the heart, veins, and arteries has been investigated. Myofibroblast is an activated fibroblast that functions as a healing mechanism in response to cardiac injury, such as myocarditis and myocardial ischemia-reperfusion injury (MIRI). However, excessive myofibroblast deposition can result in fibrosis (Baum and Duffy, 2011). Successful treatment of myocarditis using siRNA in a study by Woods et al. (2021) provides a huge discovery in the post-Covid situation because myocarditis is one of the most vulnerable cardiovascular diseases. Increased risks were recorded in all categories, including people with complete vaccine doses, one vaccine dose, and patients with a history of being infected with Covid-19 (Patone et al., 2021). Venous thrombosis and atherosclerosis are common vascular damages, with both conditions interfering with blood flow to the heart and become a highly potent factor in the development of ischemia-reperfusion injury among patients (Frank et al., 2012).

According to Dana et al. (2017), the application of siRNA therapy can degrade the complementary mRNA and interfere with the protein translation and expression of the targeted gene. Among the studies reviewed, only CD147 siRNA has been experimented on human cells, while others are animal models. Incorporation of the αSMA protein and F-actin is vital in the formation of fibrosis. Woods et al. (2021) found that CD147 siRNA caused a lack of uniformity in αSMA protein formation, thus, impaired its contractility and restrained contact with F-actin. In myocarditis treatment, the receptor for advanced glycation and products siRNA (siRAGE) suppressed the continuous synaptic action that can cause inflammation (Christaki et al., 2012). It prevented the infiltration of inflammatory cells by lowering the level of pro-inflammatory cytokine, Interleukin-6. Hou et al. (2022) and Wang et al. (2022) used vascular adhesion molecule-1 siRNA (siVCAM-1) and siRNA against MOF (siMOF) to treat ischemia-reperfusion injury, respectively. Blood restoration of ischemic tissues triggers tumour necrosis factor-α (TNF-α) and induces recruitment of neutrophils to the site. Inhibition of neutrophil adhesion by VCAM-1 siRNA prevented the inflammation of vessels (Sager et al., 2016). The application of siMOF to histone acetyltransferase KAT8 or MOF prevented excessive production of reactive oxygen species (ROS) by suppression of ROS synthesising enzyme transactivation, hence reducing oxidative stress and the inflammatory response of cardiomyocytes (Yu et al., 2018). A research by Biswas et al. (2012), the toll-like receptor 3 (TLR3) siRNA has prevented venous thrombosis by inhibiting the TLR3 signal in activating tissue factors that cause hypoxemia and fibrin deposition in lungs. In atherosclerosis, Smad2/3 siRNA supressed the Smad2/3 signalling pathway that can trigger fibrosis, induce cell apoptosis and promote the formation of fat deposition in arteries (Comarița et al., 2022).

A naked siRNA is free from chemical modification and involves the localised injection of a solution containing non-complex siRNA sequences into the targeted areas. Findings by Woods et al. (2021) and Bhagat et al. (2020) found that localised direct injection of naked CD147 and naked TLR3 siRNA on primary human fibroblast culture and mice respectively have successfully impaired the expression of targeted genes. According to Zhang et al. (2016), the presence of ribonucleases may reduce the half-life to less than 1 minute. Specific-targeting nanocomplexes have been re-designed according to the suitability of surrounding tissues and the drug composition. Endothelial cell-targeting and ROS-ultrasensitive nanocomplexes, as well as polycations macrophage-targeting nanocomplexes, have demonstrated high potential in treating cardiovascular damage in both *in vitro* and *in vivo* testing. (Hou et al., 2022; Wang et al., 2022). Extracellular vesicles can be easily found as the sources mainly originate from the body cells such as T cells and dendritic cells. According to Raposo and Stoorvogel (2013), the mechanism of the extracellular vesicle is through endocytosis, and it encapsulates the drugs in vesicles before fusing with the target tissues through integration with the plasma membrane. The encapsulation has protected the drug cargo from being off-target reactive.

The delivery of siRNAs for *in vitro* experiments is through two types of chemical transfection, which are cationic lipids and cationic polymers. The mechanism of transfection is through endocytosis followed by the release of siRNA. Lipofectamine is the most widely utilised cationic lipid because its random Brownian motion manages to avoid active transport along microtubules, hence preventing metabolic breakdown (Cardarelli et al., 2016). In active transport, siRNAs may degrade within acidic lysosomal compartments due to the long-range motion, and the solid binding perinuclear accumulation of siRNA reduces the number of plasmids transported to the nucleus of the target sites. Meanwhile, poly(L-lysine) and Poly[2 (Dimethylamino)ethyl Methacrylate] (PDMAEMA) are the most commonly used cationic polymers (Lächelt and Wagner, 2015; Englert et al., 2018). In *in vivo* studies, siRNA is administered intravenously through the tail vein since artery has very high pressure of blood flow and may cause blood loss through the punctured site. Meanwhile, subcutaneous injection is preferable when the target area is the surrounding fats. However, it has a lower rate of absorption as only a few blood vessels present in the subcutaneous layers (Kim et al., 2021).

The limitation of this review is the inclusion of minimal number of journal articles relatively, as PubMed, Scopus, and ScienceDirect databases offered a limited number of relevant studies on the delivery systems of short-interfering RNA in treating disorders of the cardiovascular system. Furthermore, the research methodologies employed in different studies are inconsistent, as some studies only performed one method, either *in vitro* or *in vivo* study. Therefore, the effectiveness of certain siRNA in treating cardiovascular damage is dependent primarily on the findings of one study approach. Additionally, only one article related to Covid-19 cases is found, which is the use of CD147 siRNA on human fibroblast culture while the other are based on animal studies.

Recommendations for future siRNA study: (1) Analysis of the method used to assess the effectiveness of the delivery system. (2) In-depth discussion on mechanism for each delivery method for comprehensive comparison between different delivery systems such as its interaction with target sites and surrounding cells. (3) Compare the effectiveness of each delivery system in both *in vivo* and *in vitro* methods, to examine whether respective delivery system is effective across cultures and living models, especially human models like human cardiovascular cell culture. (4) Provide an in-depth analysis of the correlation and significances between the route of administration and delivery method applied in treating specific cardiovascular diseases.

CONCLUSION

Considering the rising prevalence of cardiovascular damage associated with Covid-19, this review comprehensively analyses the potential of siRNA therapy as a viable solution for this critical issue. Although the findings are primarily derived from experiments on human cell culture and animal studies, they hold significant relevance. The effective siRNA delivery systems could significantly contribute to mitigating complications related to cardiovascular damage during and post Covid-19 era. Each siRNA used to treat cardiovascular damage has its own delivery system consisting of a delivery method and administration route. The method of delivery for siRNA is modified according to the sensitivity of the target site or organs. Meanwhile, a different route of administration is implemented according to the location of the target site to enhance the efficiency of the delivery method applied to fuse with the cells or release the siRNA. The delivery systems are modified through the paired combination of delivery methods and administration routes to ensure the compatibility of siRNA and target sites and silence targeted genes in both *in vitro* and *in vivo* methods without causing toxicity. While these methods have not been directly employed during the pandemic, their potential utility remains evident. Given the persistent rise in cardiovascular disease, even beyond the Covid-19 context, these findings serve as a cornerstone for future research and development in addressing the challenges posed by cardiovascular damage.

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Table 2 Effective delivery systems used in treating various cardiovascular damages

Journal Reference	Cardiovascular Damage	Type of siRNA	Method of Delivery	Study Model	Route of Administration
Woods et al., 2021	Myofibroblast	CD147 siRNA	Naked siRNA	Primary Human Fibroblast culture	Chemical transfection (Cationic lipids)
Hou et al., 2022	Ischemia reperfusion injury	Vascular cell adhesion molecule-1 siRNA (siVCAM-1)	Endothelial cell-targeting and ROS-ultrasensitive nanocomplexes comprised of PLGA NPs decorated with cRGD-poly(ethylene glycol) (PEG)-modified, ditellurium-crosslinked PEI (RPPT)	Rats	Intravenous injection
				Rat cardiac microvascular endothelial cells (RCMECs)	Chemical transfection (Cationic polymers)
Bhagat et al., 2020	Venous thrombosis	Toll-like receptor 3 (TLR3) siRNA	Naked siRNA	Mice	Intravenous injection
Kim et al., 2021	Myocarditis	Receptor for advanced glycation end products siRNA (siRAGE)	Cardiac-targeting peptide (CTP) expressing sEVs (C-sEV)	Rats	Intravenous injection
				H9C2 cells (alternative of cardiomyocytes)	Chemical transfection (Cationic lipids)
Wang et al., 2022	Ischemia reperfusion injury	siRNA against MOF (siMOF)	Polycations macrophage-targeting nanocomplexes (NCs): Man-COOH/BPAE-SS/siMOF (MBSs) NCs	Mice	Intravenous injection
				RAW 264.7 cells (mouse monocyte macrophages)	Chemical transfection (Cationic polymers)
Comarița et al., 2022	Atherosclerosis	Smad2/3 siRNA	Subcutaneous adipose tissue stem cells extracellular vesicles (EVs), or bone marrow mesenchymal stem cells EVs	Hamsters	Subcutaneous injection

***Method of study:** ☐ *in vivo* ☐ *in vitro*

Table 3 Main findings from the application of delivery systems

Method of siRNA Delivery	Effectiveness Status	Result	Reference
Naked CD147 siRNA	Effective	Arrangement of F-actin is not altered, impaired the contractility in myofibroblast as it has irregular formation of αSMA protein that prevented incorporation of F-actin and αSMA, and. Fibrosis is inhibited.	Woods et al., 2021
Endothelial cell-targeting and ROS-ultrasensitive nanocomplexes (NCs) comprised of PLGA NPs decorated with cRGD-poly(ethylene glycol) (PEG)-modified, ditellurium-crosslinked PEI (RPPT)	Effective	Inhibition of neutrophil infiltration has lessened the post effects of MIRI in both *in vitro* and *in vivo*; reduction in the size of infarcted area, muscle degeneration, cardiac fibrosis, and cardiomyocyte apoptosis.	Hou et al., 2022
Naked Toll-like receptor 3 (TLR3) siRNA	Effective	Inhibition tissue factor activity that can cause hypoxemia leading to venous thrombosis.	Bhagat et al., 2020
Cardiac-targeting peptide (CTP) expressing sEVs (C-sEV) for the delivery of RAGE-targeting small interfering RNA (siRNA) (siRAGE)	Effective	Compared to N-sEVs, the efficiency of cardiac-targeting *in vivo* are 4 folds higher while 2 folds higher through *in vitro* and no toxicity is present.	Kim et al., 2021
Polycations macrophage-targeting nanocomplexes (NCs): Man-COOH/BPAE-SS/siMOF (MBSs) NCs	Effective	Reduced the post-RI effects by alleviating the production of reactive oxygen species (ROS); cardiomyocytes degeneration, fibrosis and apoptosis.	Wang et al., 2022
Smad2/3 siRNA with subcutaneous adipose tissue stem cells extracellular vesicles (EVs) or bone marrow mesenchymal stem cells EVs	Effective	Compared to typical atherosclerosis, a significant reduction is seen in (1) The total cholesterol, triglycerides, and LDL cholesterol in arteries, (2) the thickness of arteries and (3) The expression of NF-kB transcription factor in both aortic and thoracic arteries.	Comarița et al., 2022

REFERENCES

Baum, J., & Duffy, H. S. (2011). Fibroblasts and myofibroblasts: What are we talking about? *Journal of Cardiovascular Pharmacology*, *57*(4), 376–379. https://doi.org/10.1097/fjc.0b013e3182116e39

Bhagat, S., Biswas, I., Ahmed, R., & Khan, G. A. (2020). Hypoxia induced up-regulation of tissue factor is mediated through extracellular RNA activated Toll-like receptor 3-activated protein 1 signalling. *Blood Cells Molecules and Diseases*, *84*, 102459. https://doi.org/10.1016/j.bcmd.2020.102459

Biswas, I., Garg, I., Singh, B., & Khan, G. A. (2012). A key role of toll-like receptor 3 in tissue factor activation through extracellular signal regulated kinase 1/2 pathway in a murine hypoxia model. *Blood Cells Molecules and Diseases*, *49*(2), 92–101. https://doi.org/10.1016/j.bcmd.2012.05.001

Cardarelli, F., Digiacomo, L., Marchini, C., Amici, A., Salomone, F., Fiume, G., Rogora, A., Gratton, E., Pozzi, D., & Caracciolo, G. (2016). The intracellular trafficking mechanism of Lipofectamine-based transfection reagents and its implication for gene delivery. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep25879

Christaki, E., Lazaridis, N., & Opal, S. M. (2012). Receptor for advanced glycation end products in bacterial infection. *Current Opinion in Infectious Diseases*, *25*(3), 304–311. https://doi.org/10.1097/qco.0b013e3283519b82

Comarița, I. K., Vîlcu, A., Constantin, A., Procopciuc, A., Safciuc, F., Alexandru, N., Dragan, E., Nemecz, M., Filippi, A., Chițoiu, L., Gherghiceanu, M., & Georgescu, A. (2022). Therapeutic potential of Stem Cell-Derived extracellular vesicles on Atherosclerosis-Induced vascular dysfunction and its key molecular players. *Frontiers in Cell and Developmental Biology*, *10*. https://doi.org/10.3389/fcell.2022.817180

Dana, H., Chalbatani, G. M., Mahmoodzadeh, H., Karimloo, R., Rezaiean, O., Moradzadeh, A., Mehmandoost, N., Moazzen, F., Mazraeh, A., Marmari, V., Ebrahimi, M., Rashno, M. M., Abadi, S. J., & Gharagouzlo, E. (2017). Molecular Mechanisms and Biological Functions of siRNA. *International journal of biomedical science: IJBS, 13*(2), 48–57.

Englert, C., Brendel, J. C., Majdanski, T. C., Yildirim, T., Schubert, S., Gottschaldt, M., Windhab, N., & Schubert, U. S. (2018). Pharmapolymers in the 21st century: Synthetic polymers in drug delivery applications. *Progress in Polymer Science*, *87*, 107–164. https://doi.org/10.1016/j.progpolymsci.2018.07.005

Frank, A., Bonney, M., Bonney, S., Weitzel, L., Koeppen, M., & Eckle, T. (2012). Myocardial Ischemia reperfusion injury. *Seminars in Cardiothoracic and Vascular Anesthesia*, *16*(3), 123–132. https://doi.org/10.1177/1089253211436350

Hou, M., Wu, X., Zhao, Z., Deng, Q., Chen, Y., & Yin, L. (2022). Endothelial cell-targeting, ROS-ultrasensitive drug/siRNA co-delivery nanocomplexes mitigate early-stage neutrophil recruitment for the anti-inflammatory treatment of myocardial ischemia reperfusion injury. *Acta Biomaterialia*, *143*, 344–355. https://doi.org/10.1016/j.actbio.2022.02.018

Kim, H., Mun, D., Kang, J., Lee, S., Yun, N., & Joung, B. (2021). Improved cardiac-specific delivery of RAGE siRNA within small extracellular vesicles engineered to express intense cardiac targeting peptide attenuates myocarditis. *Molecular Therapy - Nucleic Acids*, *24*, 1024–1032. https://doi.org/10.1016/j.omtn.2021.04.018

Lächelt, U., & Wagner, E. (2015). Nucleic Acid Therapeutics Using Polyplexes: A journey of 50 years (and beyond). *Chemical Reviews*, *115*(19), 11043–11078. https://doi.org/10.1021/cr5006793

Medeiros, I. G., Khayat, A. S., Stransky, B., Santos, S., De Assumpção, P. P., & De Souza, J. E. S. (2021). A small interfering RNA (siRNA) database for SARS-CoV-2. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-88310-8

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for Systematic Reviews and Meta-Analyses: the PRISMA statement. *PLOS Medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Patone, M., Mei, X., Handunnetthi, L., Dixon, S., Zaccardi, F., Shankar-Hari, M., Watkinson, P., Khunti, K., Harnden, A., Coupland, C., Channon, K. M., Mills, N. L., Sheikh, A., & Hippisley-Cox, J. (2021). Risks of myocarditis, pericarditis, and cardiac arrhythmias associated with COVID-19 vaccination or SARS-CoV-2 infection. *Nature Medicine*, *28*(2), 410–422. https://doi.org/10.1038/s41591-021-01630-0

Raposo, G., & Stoorvogel, W. (2013). Extracellular vesicles: Exosomes, microvesicles, and friends. *Journal of Cell Biology*, *200*(4), 373–383. https://doi.org/10.1083/jcb.201211138

Sager, H. B., Dutta, P., Dahlman, J. E., Hulsmans, M., Courties, G., Sun, Y., Heidt, T., Vinegoni, C., Borodovsky, A., Fitzgerald, K., Wojtkiewicz, G. R., Iwamoto, Y., Tricot, B., Khan, O. F., Kauffman, K. J., Xing, Y., Shaw, T. E., Libby, P., Langer, R., . . . Nahrendorf, M. (2016). RNAi targeting multiple cell adhesion molecules reduces immune cell recruitment and vascular inflammation after myocardial infarction. *Science Translational Medicine*, *8*(342). https://doi.org/10.1126/scitranslmed.aaf1435

Wang, Y., Hou, M., Duan, S., Zhao, Z., Wu, X., Chen, Y., & Yin, L. (2022). Macrophage-targeting gene silencing orchestrates myocardial microenvironment remodeling toward the anti-inflammatory treatment of ischemia-reperfusion (IR) injury. *Bioactive Materials*, *17*, 320–333. https://doi.org/10.1016/j.bioactmat.2022.01.026

Woods, E. L., Grigorieva, I. V., Midgley, A. C., Brown, C. V. M., Lu, Y., Phillips, A. O., Bowen, T., Meran, S., & Steadman, R. (2021). CD147 mediates the CD44s-dependent differentiation of myofibroblasts driven by transforming growth factor-β1. *Journal of Biological Chemistry*, *297*(3), 100987. https://doi.org/10.1016/j.jbc.2021.100987

Yu, L., Yang, G., Zhang, X., Wang, P., Weng, X., Yang, Y., Li, Z., Fang, M., Xu, Y., Sun, A., & Ge, J. (2018). Megakaryocytic Leukemia 1 Bridges Epigenetic Activation of NADPH Oxidase in Macrophages to Cardiac Ischemia-Reperfusion Injury. *Circulation, 138*(24), 2820–2836. https://doi.org/10.1161/circulationaha.118.035377

Zaim, S., Chong, J. H., Sankaranarayanan, V., & Harky, A. (2020). COVID-19 and multiorgan response. *Current Problems in Cardiology*, *45*(8), 100618. https://doi.org/10.1016/j.cpcardiol.2020.100618

Zhang, W., Liu, C., Bai, H., Du, G., Wang, H., Leng, H., Xu, Y., & Song, C. (2016). A Convenient In Vivo Model Using Small Interfering RNA Silencing to Rapidly Assess Skeletal Gene Function. *PLOS ONE*, *11*(11), e0167222. https://doi.org/10.1371/journal.pone.0167222