

The present and future role of ultrasound targeted microbubble destruction in preclinical studies of cardiac gene therapy

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Abstract: Multiple limitations for cardiac pharmacologic therapies like intolerance, individual variation in effectiveness, side effects, and high cost still remain, despite the recent progress in diagnosis and health support. Gene therapy is poised to be an attractive alternative in various ways for the future, refractory cardiac diseases being one aspect of it. As a novel therapy to deliver the objective gene to organs of living animals, ultrasound targeted microbubble destruction (UTMD) has therapeutic potential in cardiovascular disorders. UTMD, which binds microbubbles with DNA or RNA carriers into the shell and destroys the located microbubbles with low frequency and high mechanical index ultrasound can release target agents to specific organs. UTMD has the ability to transfect markedly through sonoporation, cavitation and other effects by way of intravenous injection that is minimally invasive and highly specific for gene deliverance. Here, we have summarized the present role of UTMD in pre-clinical studies of cardiac gene therapy which covers myocardial infarction, regeneration, ischaemia/reperfusion injury, hypertension, diabetic cardiomyopathy, adriamycin cardiomyopathy and some discussion for further studies.

Keywords: Ultrasound; microbubble; gene therapy; cardiovascular diseases (CVDs)

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Introduction

Greater incidence of cardiovascular diseases (CVDs) globally, is associated with the increase in mortality and morbidity rate every passing year (1). Despite the recent progress in diagnosis and health support, multiple limitations for cardiac pharmacologic therapy still remain, including intolerance, individual variation in effectiveness, side effects and high cost (2). Gene therapy tends to be a promising therapeutic tool and may be beneficial in refractory cardiac disease after raising insight into the molecular mechanisms of CVDs (3,4). However, experimental methods are not yet ready for clinical applications in terms of efficient delivery to the target tissue and sustained expression of transgenes.

Ultrasound targeted microbubble destruction (UTMD) is a novel therapy to deliver the objective gene to organs of living animals. It has been proven to bind non-invasively microbubbles (MBs) with DNA or RNA carriers (assemble adenoviral, plasmid or nanoparticles) into the shell and destroy the located MBs with low frequency and high mechanical index ultrasound, releasing target agents into peculiar organs (5-7). Not only can UTMD improve the transfection efficiency by several orders of magnitude, but also achieve specific target markedly (8). Due to its less invasive method and highly specific gene delivery system, UTMD is considered a promising strategy for gene therapy.

For our study the criteria and keywords of “ultrasound targeted microbubble destruction”, “gene therapy”,

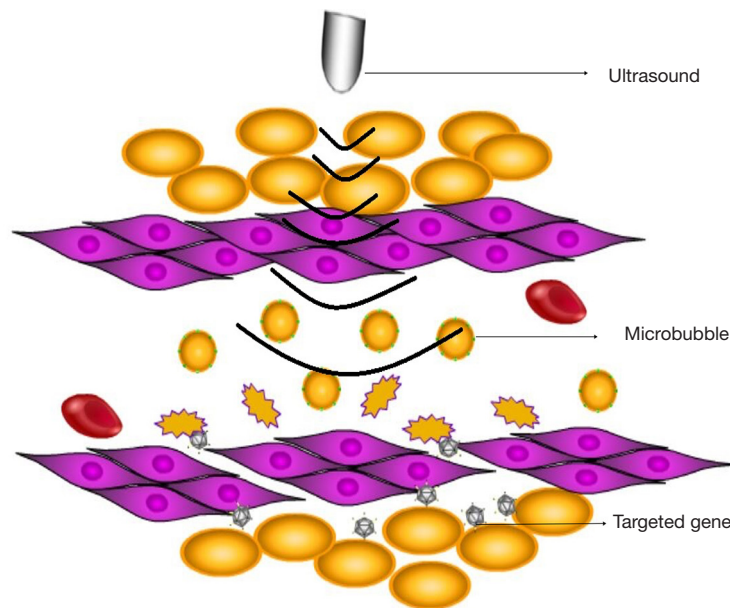


Figure 1 Therapeutic gene is released to the target cell after the microbubbles are destroyed at the site of the target tissue.

“cardiovascular diseases” was used in PubMed. All cited studies have received informed consent from each study participant and protocol approval by the ethics committee and institutional review board. This paper briefly reviews the current applications of UTMD in cardiac gene therapy and suggests avenues for further studies.

Mechanisms of UTMD

UTMD has proven to elevate the gene transfection efficiency by various preclinical studies *in vitro* and *in vivo* (9-11), as a potential target specific gene delivery tool. MBs, made of lipids, saccharide, albumin, biocompatible polymers and other materials (12-14), are considered as a promising approach for gene delivery vectors, which expands and contracts through sonoporation, cavitation and other effects.

Sonoporation, based on the specific response of the MBs upon exposure to ultrasound, is the mechanism of transferring gene into cells effectively. When exposed to ultrasound, MBs oscillate and then rupture (*Figure 1*). Not only can UTMD improve the transfection efficiency by several orders of magnitude, but also achieve specific target markedly. The nuclear membrane in the cell membrane have temporary and reversible holes (about 50 nm in diameter), allowing a molecular (weigh 10–30 KD) to get through (15). *Figure 2* shows the process of UTMD gene

delivery in tissue (16), which demonstrates the key role in augmentation of transfection efficiency (17).

Cavitation effect is another physical basis of ultrasound targeted microbubble therapy. Cell membrane permeability change is a prerequisite for gene transfection. The cavitation and mechanical effect can enhance the permeability of cell membrane, especially the cavitation effect which can be divided into; steady state and transient cavitation. The latter is a kind of strong biological effect, which can cause cell apoptosis and necrosis at the same time (18-20). It has been reported that ultrasonic cavitation effect can widen the gap between capillary endothelial cells and increase cell membrane permeability, so the microbubble or gene that is released can enter the blood vessel wall and tissue space, so as to increase the effect of targeted gene therapy.

In addition, Meijering *et al.* (21) proposed the MBs rupture resulted in hydrogen peroxide generation under ultrasound irradiation. At the same time, it caused Ca^{2+} influx and Ca^{2+} -dependent K channel opening in adjacent cell membrane, thereby causing local membrane potential hyperpolarization. The ultrasound microbubble gets into the cell by the mechanism of endocytosis and pinocytosis. Tran *et al.* (22) reported, the pressure generated by the MBs rupture induced the formation of a mechanical stimulation, activating specific channels (stretch activated channels) and non-specific ion channels, causing the exogenous molecules

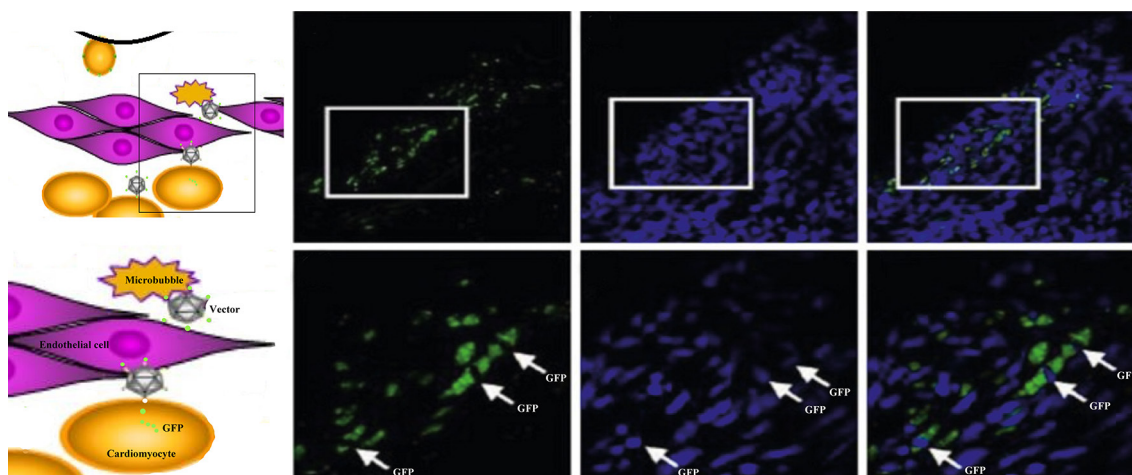


Figure 2 UTMD gene delivery in tissue. The ultrasound application leads to holes in cell membrane and capillary, which facilitates the uptake of therapeutic GFP-mark gene (16). UTMD, ultrasound targeted microbubble destruction.

(such as MBs containing transfection gene or drugs) to enter cells and play a role.

Gene delivery system in UTMD

Viral vector with high efficiency of transfection and sustained expression is the primary choice of transferring genes to the target cells in UTMD. Pre-clinical studies have proven that UTMD has synergy to combine with viral vectors and offers many benefits (23,24). First of all, transgenic expression was enhanced in the heart tissue associated with adenoviral DNA (25,26). Secondly, after injection of MBs in targeted objects loaded with the expressing luciferase or EGFP, the MBs released the site-specific strength through ultrasound irradiation that improved gene transfection efficiency (27). Also, the microbubble allows intravenous injection as delivery method as it can reduce the degradation rate of viruses by the immune system, simultaneously imposing restriction on the immune response to the viruses thus allowing intravascular administration and repetitive injections (28). Danialou *et al.* (29) investigated a 3-fold transfection of the gene and a 22-fold increase in level of expression was noted in animals treated using UTMD therapies.

Compared with viral vectors, non-viral carrier systems are potentially safer and more convenient, which can be used not only for gene application but also for direct delivery of exogenous protein. Plasmids, siRNA, miRNA and PiggyBac are common non-viral transposons for UTMD. *Table 1* shows the studies in which non-viral

vectors worked as the delivery vectors. A highly specific and minimally invasive non-viral gene delivery system is the new direction for future therapeutic procedures.

Applications of UTMD in ischemic heart disease

Myocardial infarction (MI)

Despite the stenting or bypassing of the infarcted artery, ventricular dysfunction may still progress after an extensive MI (40). Novel gene therapies may improve cardiac function by regulating gene expression, promoting tissue regeneration and regional perfusion in the infarcted myocardium (41,42). UTMD, non-invasively and selectively delivers genes to the infarct via microbubble carriers and can help to release plasmid DNA when they are targeted with an ultrasound beam. Intravenously administered lipid MBs have been proven mature in clinical evaluation of myocardial perfusion and pre-clinical cardiac gene delivery (25,43). Recently, previous pre-clinical studies have proven the potential advantages of UTMD, however it warrants pragmatic studies that still needs separate optimized protocols for different diseases. In rats, promising strategies were provided to realize the localized delivery of shRNA against PHD2 (44,45), G-CSF (46), S100A6 (47), MMP2 (31), TATp (48), SCF, SDF-1 α (30) and bFGF (49) to protect the heart from acute MI via cationic MBs. Besides, ultrasound microbubble was suggested as an effective vector for VEGF (12), SCF (13) and GDF11 (50) delivery in mice, CD151 (51) and Ang-1 (32) in rabbit, microRNA-21 (34) in swine and HGF (52) in dog.

Table 1 Non-viral carrier systems in UTMD

Vector	Study	Target gene
Plasmids	Fujii <i>et al.</i> 2009 (12)	VEGF, SCF
	Fujii <i>et al.</i> 2011 (30)	SCF, SDF-1 α
	Yan <i>et al.</i> 2014 (31)	MMP2
	Deng <i>et al.</i> 2015 (32)	Ang-1
miRNA	Kopechek <i>et al.</i> 2015 (33)	miR-23a
	Su <i>et al.</i> 2015 (34)	microRNA-21
	Liu <i>et al.</i> 2015 (35)	microRNA-21
PiggyBac	Chen <i>et al.</i> 2016 (36)	ANGPTLs
	Chen <i>et al.</i> 2015 (37)	GLP-1
	Chen <i>et al.</i> 2013 (38)	TB4
siRNA	Huang <i>et al.</i> 2016 (39)	GRK4

UTMD, ultrasound targeted microbubble destruction; Ang-1, angiopoietin-1; MMP2, matrix metalloproteinase 2; VEGF, vascular endothelial growth factor; TB4, thymosin beta 4; GRK4, G protein-coupled receptor kinase type 4.

The therapeutic value of UTMD mediated gene transfer into the infarcted heart was enhanced by these pre-clinical treatments (12,30-32,34,44-52).

Regeneration

Approximately 10% of MI patients will die, typically from ventricular arrhythmias, pump failure or myocardial rupture as reported (53). Developing strategies for regeneration of cardiomyocytes and blood vessels in the damaged area of the heart, rather than stenting or bypassing the infarcted artery has been the major goal of therapy for acute MI. Novel strategies, particularly non-invasive approaches that induce stem cell homing to the damaged heart for myocardial regeneration includes embryonic stem cells, induced pluripotent stem cells and bone marrow stem cells, which have been studied in recent years (54-56). Moreover, resident cardiac progenitor cells were discovered in the adult mammalian heart. They are self-renewing, clonogenic and multipotent, and can be differentiated into three major cardiac lineages; cardiac muscle cells, vascular smooth muscle cells and endothelial cells theoretically (57-62), despite being sparse in number. Genes such as SDF-1 (35), SDF-1/CXCR4 (63) and TB4 (38) in rat, MSC (64,65) in dog, MSC (66) in rabbit and BMSC (67) in swine were delivered directly to the heart in order to stimulate resident cardiac progenitor cells. Cardiac progenitor cells proliferate

and differentiate into three intact cardiac cell lineages after UTMD therapies. However, whether adult cardiac muscle cells can be formed from these resident progenitor cells *in vivo* still remains controversial.

Ischemia/reperfusion injury (I/R)

I/R injury is one of the main risks of heart failure, and the regenerative capacity of intrinsic stem cells plays an important role in tissue repair after injury. However, stem cells in ageing individuals have reduced regenerative potential and their tissues lack the capacity to renew. TRAF3IP2 (68), GDF11 (69), Antagomir (70), VEGF-a, IGF-1 and Cav-3 (71) in mice as well as bone marrow cell (BMC) (72), MMP2 (31) and Akt1 (73) in rat undergoing multiple application via UTMD can rejuvenate the aged heart and protect it from I/R injury.

Applications of UTMD in hypertension

Cardiac hypertrophy is induced by hypertension in pre-clinical models, but clinical translation is limited by lack of target cardiac delivery systems. Kopechek *et al.* (33) founded that UTMD mediated delivery of anti-miR-23a can suppress cardiomyocyte hypertrophy and culture cardiomyocytes in rats, laying the groundwork for future *in vivo* translational studies, which leads to targeted clinical strategies to therapeutically modulate miRNA activity in the human heart. In addition, Huang *et al.* (39) discovered the downregulation of renal GRK4 expression via UTMD lowers blood pressure in spontaneously hypertensive rats. UTMD offers a novel strategy for gene therapy in hypertension.

Applications of UTMD in cardiomyopathies

Diabetic cardiomyopathy (DCM)

DCM, one of the serious chronic complications of diabetes, is the leading cause of morbidity and mortality (74). It results in cardiac functional and structural changes, independent of hypertension, coronary artery disease, or any other known cardiac disease. The structural changes include fibrosis, apoptosis, hypertrophy of myocytes and the functional changes include systolic and diastolic dysfunction (75-80). Previous studies have confirmed the progress of cardiac dysfunction after DCM could be effectively inhibited and even reversed by gene of aFGF (81,82), bFGF (83,84) and FGF-1 (85) in rat combined with the UTMD technique.

It provides a promising strategy for DCM-targeted therapy.

Adriamycin cardiomyopathy

Adriamycin cardiomyopathy is an established lethal disease. Approximately 50% of mortality every year is figured out to be capable of maintaining heart function by stimulating adult cardiac progenitor cells to initiate myocardial regeneration when congestive heart failure develops. Lee *et al.* (86) revealed surviving gene therapy attenuates left ventricular systolic dysfunction in doxorubicin cardiomyopathy by reducing apoptosis and fibrosis, which specifically targeted the underlying biological processes in heart failure. Additionally, Chen *et al.* employed UTMD to deliver PiggyBac transposon plasmids encoding the intranuclear myocardial gene of ANGPTL8 (36) and GLP-1 (37) to rat hearts with adriamycin cardiomyopathy, which results in stimulating myocardial regeneration respectively.

To summarize, UTMD contributes a tailored approach to improve cardiac diseases. *Table 2* showed the encouraging pre-clinical studies, including applications in MI, regeneration, IR, hypertension, DCM and adriamycin cardiomyopathy. However, clinical trials have yet to produce disappointing results, possibly due to incomplete or inaccurate gene delivery (87). Early clinical trials of cell transplantation demonstrated improved perfusion, but limited cell survival may have diminished the benefits of this approach for cardiac restoration (88).

Discussion

UTMD is definitely a promising strategy to improve efficiency of cardiac gene delivery because of the low toxicity, low immunogenicity of vectors, minimum invasiveness, with the great potential for multiple application, and organs which can be targeted with its high specificity proven by increasing evidences. Pre-clinical studies have demonstrated the combination of UTMD with viral or non-viral vectors in gene delivery. UTMD not only enhances the efficiency of the viral vector, but also avoids its immunogenicity. Therefore, a novel and feasible way to support gene therapy trial for individuals with CVDs has come into effect in the past few years.

Nevertheless, future work remains to be done for the technological improvement of UTMD before clinical application (53,54). First, microbubble preparation technology needs to be optimized to efficiently carry gene

payloads while maintaining acoustic activity and prolonging circulation time to prevent clearance by the mononuclear cell as well as improving targeting techniques to enhance tissue binding force in areas of high shear stress. The illustration of optimal ultrasound parameters for each microbubble and its intended application also works (89). Second, the techniques of microbubble surface modification tend to not be mature, such as the technology to connect drugs, gene or ligand to the microbubble. The number of genes or drugs which the microbubble carries are limited to the micro vesicle transport in the blood vessels because of blood fluidity and impact resistance, which leads to short contact time between the MBs and the receptor. Also, there is shortage of formation for targeted microbubble receptor which is often below the treatment of threshold (90). Moreover, to some degree, the parameters of ultrasound influences the transfection rate of MBs. Recent studies point out the use of low frequency probe can produce a wider and more uniform sound field and that strong cavitation is the key to thrombolysis. However, the optimal parameters have not been formed yet (7). In addition, when MBs exist in the capillaries, ultrasonic irradiation can cause microvascular leakage, intracardiac hemolysis capillary rupture, bleeding, formation, inflammatory cell infiltration, myocardial cell damage and other adverse reactions (7,89,90). Therefore, more technological revolution should be taken to enhance the biological application of UTMD.

When it comes to the biological efficacy and safety of UTMD, the injured endothelial cells in part of the vessel wall, limited by toxicity as well as lack of immunogenicity and the potential for repetitive and targeted applications should be taken into account. UTMD is primarily an intravascular method of gene delivery, with the vascular endothelium being the primary target (91), while several studies were not mainly for cardiomyocyte transfection. In addition, effective application of UTMD in larger animal models is one of the major obstacles hampering UTMD application (92) and most cited studies demonstrate feasibility in rodents. Here, *Table 3* shows the specific gene delivery in target subjects, which significantly reveals the target animal models, cell types and disease states treated by UTMD. It may help to select the appropriate subjects based on the therapeutic agent. Taken together, mounting experiments with large animal models and specific target cell types as well as accurate disease states are warranted to facilitate the translation into human applications.

Taken together, the fascinating pre-clinical UTMD studies discussed here represent only a fraction among a

Table 2 Applications of UTMD in cardiac gene delivery

Disease	Study	Therapeutic	Target object	Outcome summary
Ischemic heart disease				
MI	Zhang et al. (2017)	PHD2	Rat	Localized delivery of shRNA against PHD2 protects the heart from acute myocardial infarction through ultrasound-targeted cationic microbubble destruction
	Yang et al. (2016)	CD151	Rabbit	Delivery of CD151 by ultrasound microbubbles in rabbit myocardial infarction
	Xue et al. (2016)	G-CSF	Rat	UTMD in combination with granulocyte colony-stimulating factor (G-CSF) improves cardiac function in myocardial infarction rats
	Li et al. (2016)	PHD2	Rat	UTMD enhances the PHD2-shRNA to restore myocardial function following ischemic injury in rats via improving the efficacy of therapeutic angiogenesis
	Su et al. (2015)	microRNA-21	Swine	UTMD mediated microRNA-21 transfection regulated PDCD4/NF- κ B/TNF- α pathway to prevent coronary microembolization-induced cardiac dysfunction
	Mofid et al. (2017)	S100A6	Rat	UTMD of minicircle-S100A6 attenuates infarct size and improves left ventricular systolic function and perfusion post acute myocardial infarction-reperfusion
	Liu et al. (2015)	microRNA-21	Swine	UTMD enhances gene expression of microRNA-21 in swine heart via intracoronary delivery
	Du et al. (2015)	GDF11	Mice	Repetitive targeted delivery of GDF11 by ultrasound mediated cationic microbubble destruction rejuvenates and protects the aged mouse heart
	Deng et al. (2015)	Ang-1	Rabbit	Improving the efficacy of therapeutic angiogenesis by UTMD mediated Ang-1 gene delivery to the infarcted myocardium
	Yan et al. (2014)	MMP-2	Rat	The use of MMP2 antibody-conjugated cationic microbubble to target the ischemic myocardium, enhances Timp3 gene transfection and improves cardiac function
	Zhou et al. (2013)	TATp	Rat	Synergistic effects of UTMD and TAT peptide on gene transfection: an experimental study <i>in vitro</i> and <i>in vivo</i>
	Yuan et al. (2012)	HGF	Dog	Intramyocardial injection of HGF and microbubbles in combination with insonation enhances neo-vascularization and reduces ventricular remodeling and infarct size
	Fujii et al. (2011)	SCF, SDF-1 α	Rat	Targeted ultrasound delivery of SCF and SDF-1 α genes to the infarcted myocardium recruited progenitor cells and increased vascular density
	Sheng et al. (2009)	bFGF	Rat	Basic fibroblast growth factor delivered by ultrasound-mediated destruction microbubbles for treatment of acute myocardial infarction
	Fujii et al. (2009)	VEGF, SCF	Mice	Noninvasive UTMD successfully delivered VEGF and SCF genes into the infarcted heart, increased vascular density, and improved myocardial perfusion and ventricular function

Table 2 (continued)

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Disease	Study	Therapeutic	Target object	Outcome summary
Regeneration	Li <i>et al.</i> (2015)	SDF-1/ CXCR4	Rat	UTMD improves the migration and homing of Mesenchymal stem cells after myocardial infarction by up-regulating SDF-1/CXCR4; a pilot study
	Ling <i>et al.</i> (2013)	MSC	Dog	UTMD promotes angiogenesis and heart function by inducing myocardial microenvironment change
	Chen <i>et al.</i> (2013)	TB4	Rat	Stimulation of adult resident cardiac progenitor cells by durable myocardial expression of thymosin beta 4 with ultrasound-targeted microbubble delivery
	Zhong <i>et al.</i> (2012)	MSC	Dog	Enhanced homing of mesenchymal stem cells to the ischemic myocardium by UTMD
	Xu <i>et al.</i> (2010)	MSC	Rabbit	Myocardium-targeted transplantation of mesenchymal stem cells by diagnostic UTMD improves cardiac function in myocardial infarction of New Zealand rabbits
Ischemia/ reperfusion injury	Li <i>et al.</i> (2009)	BMSC	Swine	UTMD promotes bone marrow mesenchymal stem cell transplantation for myocardial infarction
	Erikson <i>et al.</i> (2017)	TRAF3IP2	Mice	Targeting TRAF3IP2 by genetic and interventional approaches inhibits ischemia/reperfusion-induced myocardial injury and adverse remodeling
	Du <i>et al.</i> (2017)	GDF11	Mice	Targeted myocardial delivery of GDF11 gene rejuvenates the aged mouse heart and enhances myocardial regeneration after ischemia-reperfusion injury
	Kwekkeboom <i>et al.</i> (2016)	Antagomir	Mice	Increased local delivery of antagomir therapeutics to the rodent myocardium using ultrasound and microbubbles
	Chen <i>et al.</i> (2016)	BMC	Rat	UTMD enhances delayed BMSC delivery and attenuates post-infarction cardiac remodeling by inducing engraftment signals
Hypertension	Yan <i>et al.</i> (2014)	MMP2	Rat	The use of MMP2 antibody-conjugated cationic microbubble to target the ischemic myocardium, enhances Timp3 gene transfection and improves cardiac function
	Dorner <i>et al.</i> (2013)	VEGF- α , IGF-1 and Cav-3	Mice	Ultrasound-mediated stimulation of microbubbles after acute myocardial infarction and reperfusion ameliorates left-ventricular remodeling in mice via improvement of border zone vascularization
	Li <i>et al.</i> (2012)	Akt1	293FT cells/ cardiomyocytes	The effect of Akt1 gene on rat cardiomyocytes by ultrasound/microbubbles destruction
	Huang <i>et al.</i> (2016)	GRK4	Rat	Downregulation of renal G protein-coupled receptor kinase type 4 expression via UTMD lowers blood pressure in spontaneously hypertensive rats
	Kopechek <i>et al.</i> (2015)	miR-23a	Rat	Targeted delivery of an anti-mir to cardiomyocytes using ultrasound and microbubbles suppresses hypertrophy

Table 2 (continued)

Table 2 (continued)

Disease	Study	Therapeutic	Target object	Outcome summary
Cardiomyopathy				
DCM	Zhao et al. (2016)	aFGF	Rat	Prevent diabetic cardiomyopathy in diabetic rats by combined therapy of aFGF-loaded nanoparticles and UTMD technique
	Zhao et al. (2016)	bFGF	Rat	Using basic fibroblast growth factor nanoliposome combined with ultrasound-introduced technology to early intervene the diabetic cardiomyopathy
	Zhang et al. (2016)	aFGF	Rat	Advanced interfere treatment of diabetic cardiomyopathy rats by aFGF-loaded heparin-modified microbubbles and UTMD technique
	Zhao et al. (2014)	bFGF	Rat	Functional and pathological improvements of the hearts in diabetes model by the combined therapy of bFGF-loaded nanoparticles with UTMD
	Tian et al. (2013)	FGF-1	Rat	Targeted delivery of fibroblast growth factor-1 by UTMD reduces myocyte apoptosis and myocardial fibrosis and improves ventricular systolic and diastolic function in rats having DCM
DIC	Chen et al. (2016)	ANGPTLs	Rat	ANGPTL8 reverses established adriamycin cardiomyopathy by stimulating adult cardiac progenitor cells
	Chen et al. (2015)	GLP-1	Rat	Myocardial regeneration in adriamycin cardiomyopathy by nuclear expression of GLP1 using UTMD

UTMD, ultrasound targeted microbubble destruction; PHD2, prolyl hydroxylase-2; CD151, cluster of differentiation 151; G-CSF, granulocyte colony-stimulating factor; GDF11, growth differentiation factor 11; Ang-1, angiopoietin-1; MMP2, matrix metalloproteinase 2; TATp, TAT peptide; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; SCF, stem cell factor; MSC, mesenchymal stem cell; TB4, thymosin beta 4; BMSC, bone marrow mesenchymal stem cell; TRAF3IP2, TRAF3 interacting protein 2; FGF-1, fibroblast growth factor-1; GRK4, G protein-coupled receptor kinase type 4; MI, myocardial infarction; DCM, dilated cardiomyopathy; DIC, disseminated intravascular coagulation; BMC, bone marrow cell.

Table 3 Specific gene delivery in target subjects

Animal model	Target tissue/cell type	Disease state	Therapeutic agent	Study
Rat	Heart/cardiomyocyte	Myocardial infarction	S100A6	Mofid <i>et al.</i> (2017)
			TATp	Zhou <i>et al.</i> (2013)
		Ischemia-reperfusion injury	Akt1	Li <i>et al.</i> (2012)
			MMP2	Yan <i>et al.</i> (2014)
		Hypertension	miR-23a	Kopechek <i>et al.</i> (2015)
	DIC	GLP-1	Chen <i>et al.</i> (2015)	
	Heart/vascular endothelial	Myocardial infarction/DCM	bFGF	Zhao <i>et al.</i> (2016); Zhao <i>et al.</i> (2014); Sheng <i>et al.</i> (2009)
			Myocardial infarction	SCF, SDF-1 α
			G-CSF	Xue <i>et al.</i> (2016)
		Regeneration	SDF-1/CXCR4	Li <i>et al.</i> (2015).
		DCM	FGF-1	Tian <i>et al.</i> (2013)
			aFGF	Zhao <i>et al.</i> (2016)
		Hypertension	GRK4	Huang <i>et al.</i> (2016)
	Heart/cardiogenic stem cells	Myocardial infarction	PHD2	Zhang <i>et al.</i> (2017); Li <i>et al.</i> (2016)
		DIC	ANGPTLs	Chen <i>et al.</i> (2016)
Heart/cardiogenic stem cells	Regeneration	TB4	Chen <i>et al.</i> (2013)	
	Ischemia-reperfusion injury	BMC	Chen <i>et al.</i> (2016)	
Mice	Heart/cardiomyocyte	Ischemia-reperfusion injury	TRAF3IP2	Erikson <i>et al.</i> (2017)
	Heart/cardiogenic stem cells	Ischemia-reperfusion injury	Antagomir	Kwekkeboom <i>et al.</i> (2016)
		Myocardial infarction/ ischemia-reperfusion injury	GDF11	Du <i>et al.</i> (2015); Du <i>et al.</i> (2017)
	Heart/vascular endothelial	Ischemia-reperfusion injury	VEGF-a, IGF-1 and Cav-3	Dorner <i>et al.</i> (2013)
		Myocardial infarction	VEGF, SCF	Fujii <i>et al.</i> (2009)
Swine	Heart/cardiomyocyte	Myocardial infarction	microRNA-21	Su <i>et al.</i> (2015); Liu <i>et al.</i> (2015)
	Heart/cardiogenic stem cells	Regeneration	BMSC	Li <i>et al.</i> (2009)
Rabbit	Heart/cardiomyocyte	Myocardial infarction	CD151	Yang <i>et al.</i> (2016)
	Heart/cardiogenic stem cells	Regeneration	MSC	Xu <i>et al.</i> (2010)
	Heart/vascular endothelial	Myocardial infarction	Ang-1	Deng <i>et al.</i> (2015)
Dog	Heart/cardiomyocyte	Myocardial infarction	HGF	Yuan <i>et al.</i> (2012)
	Heart/cardiogenic stem cells	Regeneration	MSC	Ling <i>et al.</i> (2013); Zhong <i>et al.</i> (2012)

UTMD, ultrasound targeted microbubble destruction; PHD2, prolyl hydroxylase-2; CD151, cluster of differentiation 151; G-CSF, granulocyte colony-stimulating factor; GDF11, growth differentiation factor 11; Ang-1, angiotensin-1; MMP2, matrix metalloproteinase 2; TATp, TAT peptide; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; MSC, mesenchymal stem cell; TB4, thymosin beta 4; BMSC, bone marrow mesenchymal stem cell; TRAF3IP2, TRAF3 interacting protein 2; FGF-1, fibroblast growth factor-1; GRK4, G protein-coupled receptor kinase type 4; DCM, dilated cardiomyopathy; DIC, disseminated intravascular coagulation; BMC, bone marrow cell.

wide variety of applications in cardiac gene therapy. UTMD has a strong potential to be used as an adjuvant therapy for candidates with cardiac disorders in the future.

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Footnote

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