International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 6 -2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.06.007

In vivo imaging study of the distribution of liposoluble fluorescent drugs after epicardium *in-situ* administration by ASD device

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Abstract

Heart disease is the prime cause of mortality around the world. The treatment for these is challenging. In this experiment, we have delivered fluorescent dyes via ASD into the epicardium and by epicardial injection and to document and compare the effects of two different modes of the distribution of fluorescent dyes in vital organs of the body. We found that In vivo imaging after the administration of the fat-soluble cy5 dye for 1h (5 rats) and 24 hours (5 rats), the rat's heart was extracted by injecting a large amount of physiological saline into the ventricle. The liver, kidney, and spleen, the surface of the organ, were quickly washed with physiological saline, and the filter paper was dried. The organs were placed in a living imager to observe the distribution of fat-soluble cy5 in various tissues. Excitation wavelength: 646 nm, emission wavelength of 662 nm. Conclusively, the first time it has been proved that the distribution of liposoluble fluorescent drugs after epicardium-in-situ administration by ASD combination of VRD together with a biological stem cell-based regenerative approach turns out to be of great promise to the treatment of Heart Failure.

Keywords: Heart disease, In-vivo imaging, epicardial injection, ASD Device.

Introduction

Heart and heart failure

The heart is pumping organ of the body, which pumps the blood through the blood vessels of the circulatory system [1]. During pumping, blood provides the oxygen and nutrients and removal of metabolic wastes from the body. The heart consists of four chambers right and left atria and ventricles. The commonly right part of the heart is called the right heart, and the left part is left, heart. Blood flow in the heart is one-sided due to the heart valves, which prevent backflow. The pacemaker of the heart is the SA node, which generates impulses move through the AV node and contract the heart. The heart collects deoxygenated blood from the whole body by superior and inferior vena cava, and after oxygenation from the lungs again supplies the body through the aorta. Heart composed of three layers epicardium, myocardium, and endocardium. Cardiovascular disease (CVD) is the name for the cluster of disorders afflicting the blood

vessels and heart including hypertension, coronary heart disease (CHD), cerebrovascular disease (stroke), peripheral vascular disease, cardiomyopathy, heart attack (MI) and heart failure (HF) [2]. These clinical manifestations of HF are a primary cause of death for both women and men all over the world [3]. In 2010, CVD became the leading cause of death in developing countries [4].

Congestive heart failure

Heart failure is the condition in which abnormal cardiac function leads to an insufficient supply of blood to tissues and organs to balance their metabolic demands [5]. Heart failure often refers to congestive heart failure (CHF) is a global issue with an estimated prevalence of 40 million and is increasing parallel with the population ages. Heart failure is accompanied by various signs and symptoms including, fatigue (tiredness), swelling in the ankles, feet, legs, abdomen, and veins in the neck, shortness of breath, or trouble breathing. HF is usually diagnosed based on the patient history of signs and symptoms; it is further confirmed by various diagnostic techniques, including electrocardiography, echocardiography, various kinds of blood tests, chest radiography, etc. Traditionally HF was believed to have a group of signs and symptoms triggered by the insufficient performance of the heart muscle, and for decades numbers of therapies are investigated for HF focused on only this aspect of HF pathophysiology [5]. However, in twentieth-century multiples, pathophysiological factors including structural, functional, and biologic alterations are identified for the progression of HF [6]. So, HF is an accumulative slackening of various complications, including myocardial infarction (MI), coronary heart disease (CAD), hypertension, valvular heart disease (VHD), and cardiomyopathy; its hallmarks include hypertrophy, increased interstitial fibrosis and loss of myocytes [7].

Acute HF

Similarly, acute HF is the rapid onset of action of the CHF.

Heart failure with reduced or preserved ejection friction

Heart failure either reduced ejection fraction due to left ventricular systolic dysfunction or preserved ejection fraction due to diastolic dysfunction.

Right or left-sided HF

The right or left side of the heart not working properly may call right or left HF, respectively.

Left ventricular geometry

Generally, left ventricular geometry has been classified into four different types based on their mass, thickness, and shape. These four geometries are named as normal, concentric remodeling, eccentric hypertrophy, and concentric hypertrophy. A proper, shape, size, and mass of ventricles is very vital for the appropriate functioning of the heart. Left ventricular (LV) geometry is a significant prognosticator of HF outcomes [8]. Dilated cardiomyopathy (DCM) is the most common cause of deforming the heart geometry. Ventricular remodeling is the process by which neurohormonal, mechanical, and possibly genetic factors modify the ventricular shape, size, and function[9]. Significant hemodynamic variations in HF effect from ventricular remodeling, which is mutual in patients with chronic dysfunction of the ventricular pump and which differs by HF from patient to patient [10]. The disease initiates within the left ventricle not working properly, and the heart muscle commences dilating (stretch and become thinner). This causes the chamber of the heart to become enlarged from inside. This situation often feasts to the right ventricle and then to the atria as the disease becomes worse. When the heart chambers become dilated, the heart muscle doesn't contract ordinarily. Also, the heart can't pump sufficient blood to the demanding organ. Over time, the heart becomes weaker, and HF can occur [11]. Several changes at a molecular level, biochemical changes, and metabolic changes can take place, which is highlighted in, all this factor leads to impaired contractile performance and contributes to the progression of HF [11]. The signaling pathways of pathological cardiac hypertrophy are incredibly complicated, and beyond this topic and are reviewed in detail elsewhere [11]. Ventricular remodeling occurs for a diversity of reasons, and one out of three cases of heart problem occurs because of this reason. Myocardial infarction (MI) might be the outcome in scarring, necrosis, and thinning of the ventricular myocardium. The heart compensates for the deficiency of focal contractility by a general upsurge in wall stress and end-diastolic pressures, which may result in consequences such as a ventricular aneurysm [8].

Classification of HF

According to the New York Heart Association (NYHA) Functional, Classification HF is divided into 4 classes.

Class I: No limitation is experienced in any activities; there are no symptoms from ordinary activities.

Class II: slight, mild limitation of activity; the patient is comfortable at rest or with mild exertion.

Class III: Marked limitation of any activity; the patient is comfortable only at rest.

Class IV: Any physical activity brings on discomfort, and symptoms occur at rest.

Stages of HF

In its 2001 guidelines, the American College of Cardiology/American Heart Association (ACC/AHA) working group introduced four stages of HF.

Stage A: Patients at high risk for developing HF in the future but no functional or structural heart disorder.

Stage B: A structural heart disorder but no symptoms at any stage.

Stage C: Previous or current symptoms of HF in the context of an underlying structural heart problem, but managed with medical treatment.

Stage D: Advanced disease requiring hospital-based support, a heart transplant, or palliative care.

The ACC staging system is useful in that Stage A encompasses "pre-HF," a stage where intervention with treatment can presumably prevent progression to specific symptoms. ACC Stage B would correspond to NYHA Class I. ACC Stage C corresponds to NYHA Class II and III, while ACC Stage D overlaps with NYHA Class IV.

Current treatment options

Pharmacological treatment option

The first-line drug of choice for systolic HF is currently available classes of medications that block the renin-angiotensin-aldosterone system (RAAS) include angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARBs), aldosterone antagonists (AAs), and direct renin inhibitors (DRIs) [12]. ACE inhibitors as a first-line treatment in all patients with reduced LVSP EF ≤35-40% (NYHA I-IV) have been tested in nearly 7000 patients with systolic HF in more than 30 clinical trials. Show a decrease in hospitalization [13]. β-blocker is also the first-line drug of choice for patients who have atrial fibrillation (AF). If the patient not responds to ACE-I and ARBs or has kidney failure, then shifted to hydralazine and long-acting isosorbide dinitrate as an alternate therapy. Sometimes hydralazine and isosorbide dinitrate are also added to ACE-I and ARBs. Likewise, aldosterone antagonists are also provided benefits in addition to b-blockers or ACE-I. The second-line drug of choice is digoxin with a narrow therapeutic window, and its use is minimal due to many trails failure and used in atrial fibrillation and low blood pressure patients. Sometimes vasopressin receptors antagonists are also used as euvolemic hyponatremia.

Non-pharmacological treatment option

Lifestyle modification

Behavioral modifications and dietary guidelines play an essential role in CHF patients. Regular exercise also reduces the risk of hospital visits and improves patient quality of life.

Minimally invasive therapy

Minimally invasive therapies (MIT) like cardiac contractility modulation (CCM) are used in patients with moderate to severe heart failure NYHA II-IV classifications. Automatic implantable cardioverter-defibrillator (AICD) is used in patients with reduced ejection fraction < 35%, ventricular tachycardia, or sudden cardiac death. Pacemaker and ICD are used for patients with reduced ejection fraction < 35% and average QRS complex. Biventricular pacing like cardiac resynchronization therapy (CRT) is also used in patients with prolonged QRS complex when added to standard medication therapy.

Surgical therapies (Ventricular assist therapy)

Patients with severe HF are candidates for ventricular assist therapy (VAD) or total artificial heart (TAH) as a bridge to transplant (BTT) or destination therapy (DT) for advanced heart failure. In particular cases, heart transplantation is possible but must be in a safe zone from rejection.

Palliative care

Patients with CHF have shortness of breath and chest pain, and palliative care is not the last resort for such kind of patients. There is no other treatment option except cardiac transplant, and a growing number of patients with stage IV are considered for palliative care according to the ACC/AHA guidelines. A 2017 review found that palliative care is associated with improved outcomes, such as QOL, symptom burden, and satisfaction with care.

Biological treatment

Tissue engineering is the understanding principles of tissue growth and applying this to produce functional replacement tissue for clinical use [14]. It can overcome the lacuna exiting in the current interventional and pharmacological therapies and heart transplantation. Different cell delivery systems were considered, including direct injection or infusion of the cells, which led to high leakage to surrounding tissues, non-homogeneous cell distribution, with consequent poor reproducibility, and disrupted ECM and poor survival. All the drawbacks were overcome with the patch delivery direct application, which appears to offer extensive and homogeneous area coverage of the heart. Using scaffolds for cardiac regenerative medicine has a rather long history. Heart patches are currently being under active investigation and use to provide structural support and delivery of therapeutic agents. Therapeutic agents, including drugs, proteins, stem cells, and growth hormones, can be seeded on it for various applications due to the porous nature of the scaffold[15]. Here we will discuss selected heart patches that are under investigation.

Cardio - supportive devices (Ventricular reconstruction therapy)

There are various surgical and interventional device therapies investigated for the management of HF. Since that ventricular Reconstruction therapy was born and several new development and improvement take place in this arena, parallel with time and demand. Among surgical procedures for ventricular restoration comprises of Batista procedure [16] and Dor procedure [17]. The Dor procedure resulted in developed LV geometry and functional outcomes [17]. Though numerous randomized studies of ventricular reconstruction didn't show a change in the primary endpoint, therefore, it has been heavily criticized [18]. The Myosplint was an implantable device designed for use in the treatment of CHF resulting from severe LV dilatation by modifying the geometry of the LV. The Coapsys device was used as a filament that purposed to reconstruct the papillary muscles. The Coapsys device was a specific refinement of the original Myocor Myosplint. RCTs with 165 patients were performed but stop prematurely due to funding [19]. However, there was a significant improvement in the survival rate of patients treated with Coapsys device compared with bypass and mitral valve annuloplasty. The internal percutaneous device, which is the most reported, is "Percutaneous left ventricular partitioning device targeting anterior-apical scars" (Parachute device). It consists of an apex ocular, which is delivered through a retrograde transfemoral method, passed by the aortic wall, and the left ventricle apex. It is composed of a balloon and nitinol frame attached to the ventricle apex, which classifies the ventricle into the static and dynamic chamber [20]. A study with 39 patients showed an 88% suitability rate and 90% procedural success [21]. The results demonstrate its clinical significance in terms of improvement in functional class, ejection fraction (EF), end-diastolic, and systolic volume. Revived TC system uses off-pump surgical isolation to isolate the LV side of the aneurysm from the wall to the septum, a study conducted on 26 patients in 2012 show improvement in EF, LVESV, QOL, and the 6MWT (six minutes' walk test) [22], another clinical trial for ischemic cardiomyopathy is ongoing and expected to complete in early 2019. Besides therapies mentioned above, there are large numbers of therapies that are under investigation for almost one century for LV reconstruction is Direct Cardiac Compression (DCC) devices and Ventricle Restraint Therapy (VRT). This progressive phenomenon is clinically evident, measurable, and amenable. To reduce the myocardial oxygen consumption (mVO2), wall stress, and adverse remodeling, in ventricles, restraint therapy developed. VRT refers to the treatment approach for HF by the supportive role of various biocompatible materials from outside the heart muscle without having direct contact with the blood. The main aim of VRT is to provide structural support and to prevent further left ventricles (LV); this therapy can achieve remodeling. Several kinds of devices and procedures were investigated in this arena and are even studied at different clinical phases.

The heart is a muscular organ in most animals, which pumps blood through the blood vessels of the circulatory system. Blood provides the body with oxygen and nutrients, as well as assisting in the

removal of metabolic wastes. In humans, the heart is located between the lungs, in the middle compartment of the chest.

In humans, other mammals, and birds, the heart is divided into four chambers: upper left and right atria; and lower left and right ventricles. Commonly the right atrium and ventricle are referred together as the right heart and their left counterparts as the left heart. Fish, in contrast, have two chambers, an atrium, and a ventricle, while reptiles have three chambers. In a healthy heart, blood flows one way through the heart due to heart valves, which prevent backflow. The heart is enclosed in a protective sac, the pericardium, which also contains a small amount of fluid. The wall of the heart is made up of three layers: epicardium, myocardium, and endocardium.

The heart pumps blood with a rhythm determined by a group of pacemaking cells in the sinoatrial node. These generate a current that causes contraction of the heart, traveling through the atrioventricular node and along with the conduction system of the heart. The heart receives blood low in oxygen from the systemic circulation, which enters the right atrium from the superior and inferior vena cava and passes to the right ventricle. From here, it is pumped into the pulmonary circulation through the lungs where it receives oxygen and gives off carbon dioxide. Oxygenated blood then returns to the left atrium, passes through the left ventricle, and is pumped out through the aorta to the systemic circulation where the oxygen is used and metabolized to carbon dioxide. The heart beats at a resting rate close to 72 beats per minute. Exercise temporarily increases the rate, but lowers resting heart rate in the long term, and is suitable for heart health.
Heart failure is a severe condition, and usually, there's no cure. But many people with heart failure lead a full, enjoyable life when the situation is managed with heart failure medications and healthy lifestyle changes. It's also helpful to have the support of family and friends who understand your situation. Doctors usually classify patients' heart failure according to the severity of their symptoms. The table below describes the most commonly used classification system, the NYHA Functional Classification [23]. It places patients in one of four categories based on how much they are limited during physical activity. No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath). Slight limitation of physical activity comfortable at rest. Regular physical activity results in fatigue, palpitation, dyspnea (shortness of breath) (Marked limitation of physical activity, comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea. Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases. No objective evidence of cardiovascular disease. No signs and no limitation in ordinary physical activity. Objective evidence of minimal cardiovascular disease. Mild signs and slight weakness during ordinary exercise. Comfortable at rest [6]. Objective evidence of moderately severe cardiovascular disease. Marked limitation inactivity due to symptoms, even during less-than-ordinary exercise. Comfortable only at rest [7]. Objective evidence of severe cardiovascular disease severe limitations. Experiences symptoms even while at rest. Liposoluble (comparative more liposoluble, superlative most liposoluble) Soluble in lipids (or in organic solvents). Synonym: fat-soluble. The pericardium is a double-walled sac containing the heart and the roots of the great vessels. The pericardial sac has two layers, a serous layer, and a fibrous layer. It encloses the pericardial cavity, which contains pericardial fluid.

The pericardium fixes the heart to the mediastinum, gives protection against infection, and provides the lubrication for the heart. The pericardium is a tough double-layered fibro serous sac that covers the heart. The space between the two layers of serous pericardium (see below), the pericardial cavity, is filled with serous fluid, which protects the heart from any kind of external jerk or shock. There are two layers to the pericardial sac: the outermost fibrous pericardium and the inner serous pericardium. The fibrous pericardium is the most superficial layer of the pericardium. It is made up of dense and loose connective tissue, which acts to protect the heart, anchoring it to the surrounding walls, and preventing it from overfilling with blood. It is continuous with the outer adventitial layer of the neighboring great blood vessels. The serous pericardium, in turn, is divided into two segments, the parietal pericardium, which is fused to and inseparable from the fibrous pericardium, and the visceral pericardium, which is part of, or in some textbooks synonymous with, the epicardium. Both of these layers function in lubricating the heart to prevent friction during heart activity.

The visceral layer extends to the beginning of the great vessels (the large blood vessels serving the heart), becoming one with the parietal layer of the serous pericardium. This happens in two areas: where the

aorta and pulmonary trunk leave the heart and where the superior vena cava, inferior vena cava, and pulmonary veins enter the heart. In between the parietal and visceral pericardial layers, there is a potential space called the pericardial cavity, which contains a supply of serous lubricating fluid known as the pericardial fluid.

When the visceral layer of serous pericardium comes into contact with the heart (not the great vessels), it is known as the epicardium. The epicardium is the layer immediately outside of the heart muscle proper (the myocardium). The epicardium is largely made of connective tissue and functions as a protective layer. During ventricular contraction, the wave of depolarization moves from the endocardial to the epicardial surface. Anatomical relationships-

(i) Surrounds heart and bases of pulmonary artery and aorta.
(ii) Deep to the sternum and anterior chest wall.
(iii) The right phrenic nerve passes to the right of the pericardium.
(iv) The left phrenic nerve passes over the pericardium of the left ventricle.
(v) Pericardial arteries supply blood to the dorsal portion of the pericardium

Function

(i) It sets the heart in the mediastinum and limits its motion.
(ii) Protects it from infections coming from other organs (such as lungs)
(iii) Prevents excessive dilation of the heart in cases of acute volume overload.
(iv) Lubricates the heart.

Inflammation of the pericardium is called pericarditis. This condition typically causes chest pain that spreads to the back that is worsened by lying flat. In patients suffering from pericarditis, a pericardial friction rub can often be heard when listening to the heart with a stethoscope. Pericarditis is usually caused by a viral infection (glandular fever, cytomegalovirus, or coxsackievirus), or more rarely with a bacterial infection, but may also occur following myocardial infarction. Pericarditis is usually a short-lived condition that can be successfully treated with painkillers, anti-inflammatories, and colchicine. In some cases, pericarditis can become a long-term condition causing scarring of the pericardium, which restricts the heart's movement, known as constrictive pericarditis. Constrictive pericarditis is sometimes treated by surgically removing the pericardium in a procedure called a pericardiectomy.

Fluid can build up within the pericardial sack, referred to as a pericardial effusion. Pericardial effusions often occur secondary to pericarditis, kidney failure, or tumors and frequently do not cause any symptoms. However, large effusions or effusions that accumulate rapidly can compress the heart in a condition known as cardiac tamponade, causing breathlessness and potentially fatal low blood pressure. Fluid can be removed from the pericardial space for diagnosis or to relieve tamponade using a syringe in a procedure called pericardiocentesis. For cases of recurrent pericardial effusion, an operation to create a hole between the pericardial and pleural spaces can be performed, known as a pericardial fenestration. Transcatheter atrial septal defect (ASD) closure is now a widely recognized alternative to surgical closure for suitable secundum ASDs. Closure of a large ostium secundum ASD (OS-ASD), which measured 40 mm on trans-esophageal echo (TEE) and was closed with a 46 mm device. (Lifetech Scientific Inc., China). To the best of our knowledge, this is the largest size of the device used for closure of ASD to date. Atrial septal defect (ASD) is a heart defect in which blood flows between the atria (upper chambers) of the heart. Usually, after PFO closure, the atria are separated by a dividing wall, the interatrial septum.

ASD Device

An active hydraulic ventricle supporting the drug delivery system (ASD) consists of highly biocompatible silicon-based [24] fistulous net cover surrounding both the ventricles as communicating entirely with each other or form a plurality of independent areas, and the interior of each separate area is intercommunicating. The interconnecting tubules have some intentionally designed apertures at the internal surface, to generate communications of materials or signals between the heart and the net cover. Then the net cover can be connected to an administrating system outside the body via the ends connected to the exterior of the institution Using ASD device, the fluid could deliver BMSCs inside the tubes, and it could directly affect cardiomyocytes [25, 26], by producing direct biological action on the cardiac muscle or other heart tissues without entering the blood circulation. A variety of therapeutic materials can be filled from the outside, spread all around the net cover, and then applied to the ventricle

via the apertures [4]. The diameter of the hollow tubules and apertures are most important in the smooth delivery of therapeutic agents. When the width of the apertures is not larger than half of the diameter of the hollow tube, the hollow tube performs best. And if the diameter of the apertures is too large, the effect of the hollow tubes will be reduced. Particularly, the diameter of the hollow tubes is 1-2 mm, wherein the diameter of the apertures is 0.5-1.0 mm. Tubes of diameter within this scope have an excellent support effect on the heart wall, good hydraulic reactivity, and excellent permeation efficiency to the heart tissue for the medicine [27]. The size of the ASD device is different for different animals; the size for SD rats has been; accordingly, the species, concentration, a dose of stem cells, and the speed of administration can be well adjusted outside the body. ASD device implantation After the confirmation of HF, the pericardium was exposed, and the ASD device was placed around the heart ventricles, which was achieved by sliding the device over the epicardium, up to the level of the atrioventricular (AV) junction **(Fig. 2c).** After achieving the correct position on the heart, it was then stitched with AV junction by prolene sutures [28]. The ASD device was connected to an implantable catheter, and ASD portacath was tunneled subcutaneously through the second intercostals space into the left anterior chest wall and was extended outside the body through the 1 cm opening made in the skin at spinotrapezius Chest retractor was withdrawn, and the ribs were rejoined together by discontinuous stitching. After the placement of the ASD device, rats were divided into 2 experimental groups (n = 6), namely HF + ASD and HF + ASDBMSCs groups. Postoperatively, animals received penicillin sodium for antibiotic prophylaxis (5 wU/100 g i/m every 24 h for 5 days).

Functions of ASD device

Active hydraulic ventricular Support Drug delivery system (ASD) device can provide 1) Physical support 2) Biological and pharmacological agents delivery 3) Real-time heart monitoring. ASD is a mesh-like device consisting of several hollow tubules, which surrounds both ventricles of the heart, as shown in **Fig. 1**. All the hollow tubes are completely interconnected to form a plurality of independent areas, and the interior of each separate area is intercommunicating, while the separate areas are not communicating. No matter with or without the apertures, each of the separate regions forming the net cover has two or more ends connected to the exterior of the body, and the diameter of the apertures is not larger than a half of the diameter of the hollow tubes. The hollow ASD tubules can be filled with various kinds of liquid of different physical characteristics, and then the corresponding reaction pressure generated can be applied to the ventricle and the surfaces of the heart, as shown in **Fig. 1B**. A pneumatic pump can be attached externally with ASD tubules, which can deliver an adjustable and measurable optimized restraint at the beginning of therapy, as well as the heart shrinks during active reverse remodeling. We hypothesize that ASD will attenuate LV remodeling and improve heart performance. The system also can be incorporated with the local administration of biological and therapeutic agents. A semi-permeable membrane can be attached with the apertures of ASD tubules for selective delivery to control the size, structure, and permeation flow rate of the drug molecules applying on the heart epicardium, as shown in **Fig. 1C**. We believe that ASD has the potential to deliver drugs to the heart with more control and in a precise manner. Similarly, ASD has the ability to offer drugs like SM locally to the epicardium of the heart. The gold standard building material of ASD is silicon, very common, highly biocompatible, and immunogenic material and the wall from other materials of macromolecule and flexible physical characters, selected from nanophase materials capable of loading and releasing the medicine.

Aim of this study:

We are proposed to deliver fluorescent dyes via ASD into the epicardium and by epicardial injection and to document and compare the effects of two different modes of the distribution of fluorescent dyes in vital organs of the body animals; the size for SD rats has been shown; accordingly, the species, concentration, a dose of stem cells, and speed of administration can be well adjusted outside the body.

Materials and Methods

Fat-soluble fluorescent dye Cy5-NHS, BL-420 biological function signal acquisition system, HX-300s animal ventilator, live animal imager, surgical instrument set (including scissors, scalpel, tissue scissors, ophthalmic scissors), ophthalmology, tissue sputum, straight curved hemostats, straight bend mosquitoes, hemostats, etc.), syringes.

Experimental animals

A total of 20 SD rats of 200-250 g were randomly divided into two groups: group A: ASD for dye group; group B: epicardial injection for dye group.

(1) Anesthesia was administered by intraperitoneal injection with 10% chloral hydrate at a dose of 0.3 ml/100 g. The rat is placed in the supine position on the operating table to remove the neck and chest coat.

(2) Intubation of the trachea: the trachea was separated, the rats were intubated, and the intubation was connected to the ventilator to maintain the respiration of the rats.

(3)Open the chest with ASD, give the dye: Group A rats do all the mouth in the chest and open the chest in the 3, 4 intercostal spaces, tear the pericardium, and implant ASD. The fat-soluble cy5 dye (0.1 mg/kg) was administered through the ASD line.

(4) Epicardial injection to the dye: Group B rats do all the mouth in the chest, open the chest through the 3, 4 intercostal space, and slowly inject the fat-soluble cy5 dye (0.1 mg/kg) into the pericardium.

Designing of ASD

The present invention relates to a device for treating various kinds of heart diseases, more particularly for treating heart failure or various kinds of cardiomyopathies. Active hydraulic ventricular support drug delivery system (ASD device) [27] can provide 1) Physical support 2) Biological and pharmacological agents delivery 3) Real-time heart monitoring. ASD is a mesh-like device consisting of several hollow tubules, which surrounds both ventricles of the heart, as shown in **Fig. 1.** All the hollow tubes are completely interconnected to form a plurality of independent areas, and the interior of each independent area is intercommunicating, while the independent areas are not communicating. No matter with or without the apertures, each of the independent regions forming the net cover has two or more ends connected to the exterior of the body, and the diameter of the apertures is not larger than half of the diameter of the hollow tubes. The hollow ASD tubules can be filled with various kinds of liquid of different physical characteristics, and then the corresponding reaction pressure generated can be applied to the ventricle and the surfaces of the heart, as shown in **Fig. 1B**. A pneumatic pump can be attached externally with ASD tubules, which can deliver an adjustable and measurable optimized restraint at the beginning of therapy, as well as the heart shrinks during active reverse remodeling. We hypothesize that ASD will attenuate LV remodeling and improve heart performance. The system also can be incorporated with the local administration of biological and therapeutic agents. A semi-permeable membrane can be attached with the apertures of ASD tubules for selective delivery to control the size, structure, and permeation flow rate of the drug molecules applying on the heart epicardium, as shown in **Fig. 1C.** We believe that ASD has the potential to deliver drugs to the heart with more control and in a precise manner. Similarly, ASD can deliver drugs like SM locally to the epicardium of the heart. The gold standard building material of ASD is silicon[29], very common, highly biocompatible and non-immunogenic material and the wall from other materials [30] of macromolecule and flexible physical characters, selected from nanophase materials capable of loading and releasing the medicine. The safety of biomaterials is important for successful drug delivery without any complications. Leak testing (Cincinnati Test Systems Shanghai) was performed to ensure any leakage and blockade in ASD tubules. First of all 3D model of ASD was designed by Using Rhinoceros 5.0 software (Robert and Mc Neel, USA) then a blue wax model was developed by Nanjing Shining 3D Tech Co., Ltd., Nanjing, China. A prepared silicon solution was filled in a wax model, which was then cured by heating at 50°C for 1 hour. Next, the assembly was subjected to a temperature of 100 for 30 minutes to let the wax melt and get a pure silicone ASD device, as shown in **Fig. 2.** In the present study, the ASD tubules were punched to make apertures for the delivery of SM by using laser beam DNUVM8 (Nanjing DiNai laser Sci. and Tech, Co., Ltd., Nanjing, China) as shown in **Fig. 1C.** ASD has four tubules like appendages among these four tubules; two were extended for *Salvia miltiorrhiza* delivery in SD rats, as shown in **Fig. 2. C.** Dimension of the ASD device is different for different experimental animals. In this study, the dimensions of ASD for SD rats are described in **Fig. 1.A.**

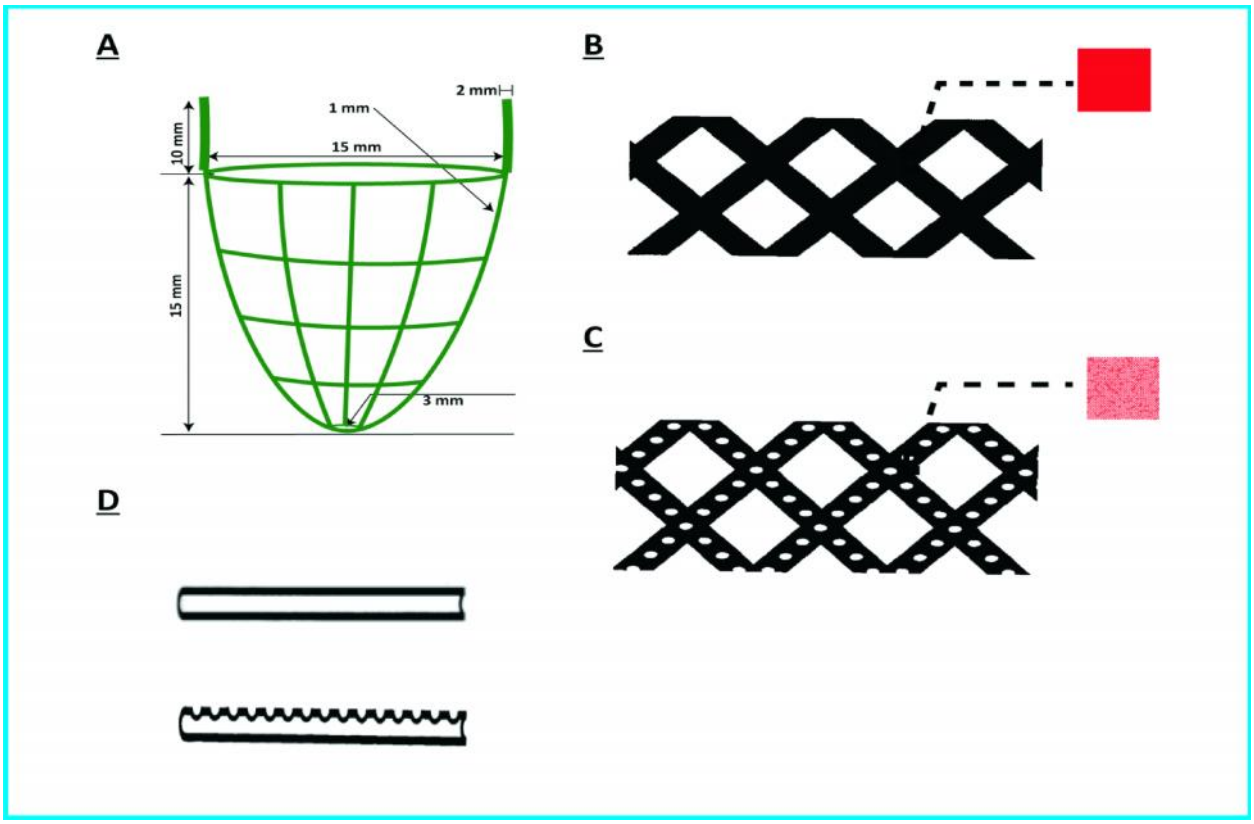

Figure 1. Different view angles of ASD.**A.** Dimensions of ASD**, B.** Outer and inner lining of tubules, **C.** Inner lining of tubules, **D.** Cross-section area of tubules [4].

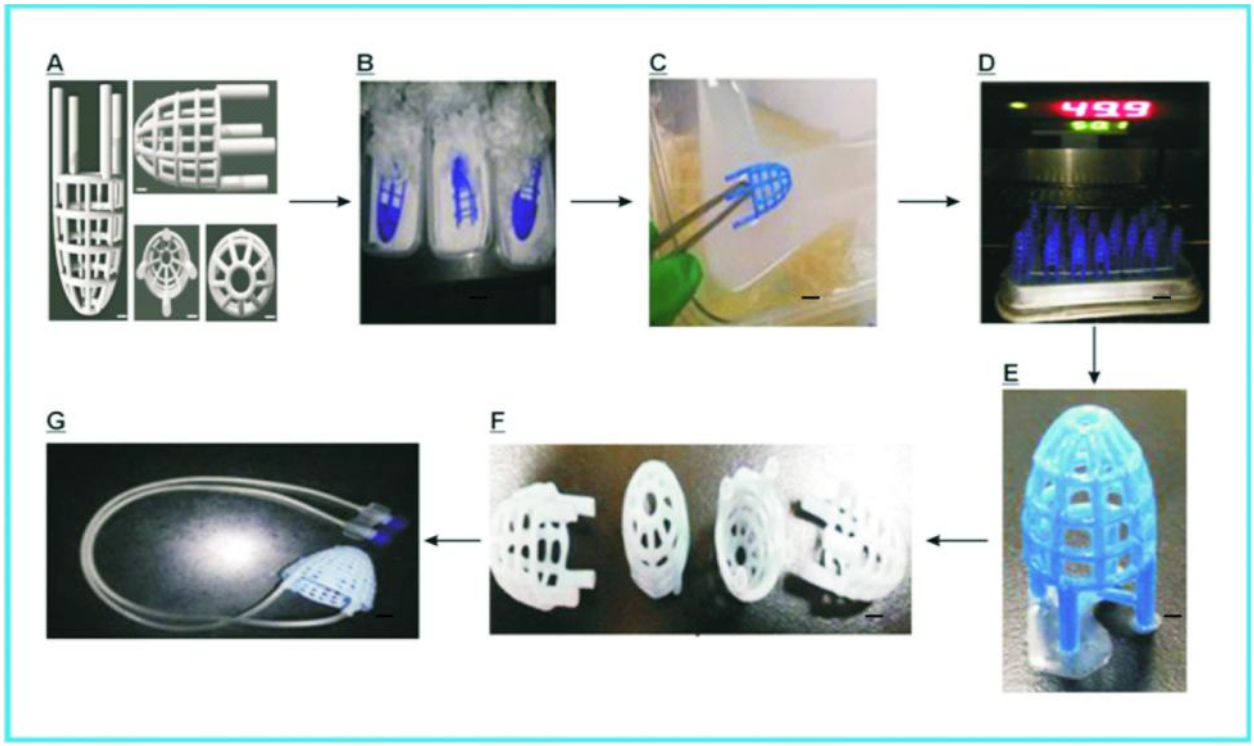

Figure 2. ASD fabrication **A.**3-D model of ASD, **B.** 3-D blue wax model, **C.** Wax model of ASD was plunged into liquid silicon, **D.** Model put into the oven for 1hour at 50°C, **E.** Blue wax start melting from the ASD model at 100°C for 30minutes, **F.** ASD from pure silicon, **G.** ASD connected with an implantable catheter, scale bar 100µm [4].

ASD device implantation

After the confirmation of HF, the pericardium was exposed, and the ASD device was placed around the heart ventricles, which is achieved by sliding the device over the epicardium, up to the level of the atrioventricular (AV) junction, After obtaining the correct position on heart it was then stitched with AV junction by prolene sutures (4-0) [31]. The ASD device was connected to an implantable catheter, and ASD portacath was tunneled subcutaneously through the second intercostals space into the left anterior chest wall and was extended outside the body through the 1 cm opening made in the skin at spinotrapezius as shown in Chest retractor was withdrawn, and the ribs were rejoined together by discontinuous stitching. After the placement of the ASD device, rats were divided into 2 experimental groups (n=6), namely HF + ASD and HF + ASDBMSCs groups. Postoperatively, animals received penicillin.

Results

In vivo imaging: After the administration of the fat-soluble cy5 dye for 1h (5 rats) and 24 hours (5 rats), the rats were extracted from the organs of the rats by injecting a large amount of physiological saline into the ventricle. The liver, kidney, and spleen, the surface of the organ, were quickly washed with physiological saline, and the filter paper was dried. The organs were placed in a living imager to observe the distribution of fat-soluble cy5 in various tissues. Excitation wavelength: 646 nm, emission wavelength of 662 nm.

Figure 3. The first image is a scale that shows the intensity of fluorescence represented by each color in a living icon.

There is a value on the right side of the ruler. The larger the value is, the stronger the fluorescence intensity will be.

The name at the bottom of the image represents the group and number of animals used in the experiment. Asd6-脂溶性cy5-24h represents the picture taken after 24 hours of the death of rat no. 6 given lipid-soluble fluorescent dye through ASD. If the number is 心外膜7- 脂溶性cy5-1h, it represents the picture taken by rat no. 7, who was assigned lipid-soluble fluorescent dye through the epicardial membrane and sacrificed after 1 hour. The second row of living imaging images is the superposition of the second and third pictures in the first row. In the writing of the article, you can only present the color pictures similar to the second row to illustrate the experimental results. The three pictures in the second row are familiar; you can choose one for analysis. *In vivo* imaging showed that lipid-soluble fluorescent dyes were mostly distributed in the heart, which was obtained by comparing the color of the heart with that of other organs in the scale of the first picture. Finally, you can make a comparative statistical analysis of the effects of different time on the distribution of fluorescent dyes, as well as the effects of varying dosing methods on dye distribution.

Discussion

Despite the use of existing drugs for optimal treatment in end-stage heart failure, the overall effectiveness of these therapeutic approaches is limited as well as possible lethal side effects must be considered [32] to attain desired therapeutic results. Therefore, novel therapeutic strategies are imperative, which have the potential to combat multiples etiology. Not only the successful delivery of therapeutic drugs at the target site (with minimal or no side effects), but also the biosafety of these therapeutics must be considered. In the present study, ASD was used, which is made from silicon, a highly biocompatible material, and is the best option for safe delivery of many therapeutic drugs to the heart muscle [32] without causing any significant side effects. The mesothelium cells in the heart, which forms the epicardium, actively transport cells, fluids and they can synthesize and secrete different inflammatory mediators, and these mediators respond to an external stimulus that plays essential roles in the regulation of different responses like inflammatory, immune and tissue repair [33]. ASD is an innovative therapeutic device that not only successfully delivers drugs to the pericardium of the heart to treat heart failure but also has a supportive action, which helps in further preventing dilatation and attenuation of LV remodeling. Furthermore, ASD can temporally deliver the drug and maintain a therapeutic level of medicine to the heart. Interestingly, ASD also has a permeable membrane with the small pores, which help in selected drug delivery to the heart muscles.

The traditional epicardial drug delivery system is a patch of matrix and medicines. It is attached to the ventricular surface of the heart and releases the drug slowly to prevent ventricular arrhythmias due to myocardial infarction, heart failure, or post-cardiac surgery. Delivery of drugs directly to the epicardium can minimize the toxicity and systemic side effects. ASD provides a new way for the administration of drugs with high general toxicity. A significant advantage of ASD devices is that the medicines administer through ASD can reach everywhere of the ventricles. Besides, the drug can be delivered anytime through ASD.

Moreover, it also provides the benefit of changing the drug dose according to the patient's condition at any time, because the external tubes of ASD are elicited from the chest to the outside. Furthermore, ASD can load with BMSCs to treat myocardial fibrosis and promote angiogenesis. Previously, our laboratory has confirmed that the administration of BMSCs released on the epicardium through ASD could reverse heart failure in rats. ASD could deliver drugs or cells repeatedly for treating acute (e.g., ventricular arrhythmias) and chronic heart diseases (e.g., chronic heart failure and myocardial infarction) and provide physical support to the heart.

This study demonstrated that ASD is a comprehensive therapeutic platform for treating heart diseases. There are certain limitations, and further research is required to advance this technology along the translational path. First, the change of epicardial potential was not recorded because of the small heart size of the rat. Second, we did not measure the serum concentration and myocardial concentration of lidocaine because the primary goal of the study was to evaluate the feasibility and effectiveness of this novel device. Third, ASD should be designed for each animal individually, so that it will fit the heart better.

The results of this study guarantee that drug delivery with new ventricular restraint therapeutic device (ASD) could be a promising strategy for the treatment of heart failure. They are delivering the drug through ASD as combination therapy is a smart therapeutic option. Present work could prove that combination therapy is a useful strategy to treat major diseases like heart failure than single drug administration.

Conclusion

In summary, herein, for the first time, it has been proved that the distribution of liposoluble fluorescent drugs after epicardium-in-situ administration by ASD combination of VRD together with biological stem cells based regenerative approach turns out to be of great promise to the treatment of HF. The constructed therapeutic platform for the management of advanced HF exhibits multiple therapeutic functions in a single action, by considering that multiple therapeutic materials, like stem cells, drugs, and diagnostic material could be delivered in a well-controlled way. Future efforts should be focused on equipping the ASD with more bidirectional biosensors to make the device more powerful, not only for the relevant disease treatment but also for the effective diagnosis.

Acknowledgments

This study is sponsored by the National Nature Science Foundation of China (No.30973003 & 30901993) and Qing Lan Project of Jiangsu Province,

China. Furthermore, we are highly thankful to the Naveed M., (Malik, China Pharmaceutical University) for critically reviewing the text of this paper.

Conflict of interest

All authors declare no conflict of interest

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DOI:10.22192/ijarbs.2019.06.06.007	

How to cite this article:
Ashikujaman Syed, Cunyu Li, Zhou Xiaohui. (2019). *In vivo* imaging study of the distribution of liposoluble fluorescent drugs after epicardium *in-situ* administration by ASD device. Int. J. Adv. Res. Biol. Sci. 6(6): 58-70.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.06.007