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Two-Year Heart Failure Study with Allogeneic Myoblast Transplantation

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Abstract

Objectives: Allogeneic myoblast transplantation (AMT), cyclosporine immunosuppression and coronary artery bypass grafting (CABG) were used to treat end-stage heart failure (HF) subjects without hope of obtaining a heart transplant. **Background:** Severe myocardial infarction conveys serious complications such as ventricular aneurysm, wall thinning and rupture with fatal consequences. **Methods:** After meeting Inclusion/Exclusion criteria and signing Patient Informed Consents, 10 HF subjects having mean thinnest wall thickness of 2.21 ± 0.55 mm and ventricular aneurysms were admitted under intensive care. Each subject took daily cyclosporine for three weeks. On the third day of cyclosporine administration, approximately 1 billion myoblasts were implanted through 20 injections into the infarcted myocardium following CABG. **Results:** Safety No subject suffered death, viral infection, malignant arrhythmia, reduction in cardiac output, immune rejection, or aneurysm growth. No significant difference was found before versus after treatment in the mean levels of blood routine, liver and kidney enzymes, electrolytes and fibrinogen. Efficacy Emission computed tomography (ECT) and magnetic resonance (MR) demonstrated significant increases in viability and perfusion. Mean left ventricular ejection fraction (LVEF) significantly increased ($P < 0.05$) by 20.1% and 19.3% at 6 months and at 2 years postoperatively. New York Heart Association (NYHA) class improved by 2 grades, including 6-minute walk test (6 MWT) distance increase, and reductions in the number of episodes of angina pectoris, chest tightness, shortness of breath after exercise, and nighttime sit-up breathing. **Conclusions:** For the first time, AMT in adjunct use with CABG and cyclosporine demonstrated that cell survived and engrafted in patients with ischemic cardiomyopathy; in this small study the cell transplant

was safe. The improvement in heart function and quality of life could be secondary to combined effect of bypass and cell transplant. A larger randomized clinical trial is required to confirm the efficacy.

Keywords

Heart Failure, Allogeneic Myoblast Transplantation

1. Introduction

Heart muscle degeneration is a leading cause of debilitation and death in humans. Its primary cause is partial or complete obliteration of the coronary artery, often precipitating acute myocardial infarction (AMI). Long-term ischemia leads to ventricular cell death and myocardial necrosis. Although medication, transmyocardial revascularization (TMR), and CABG can increase local blood supply and reduce short-term mortality and morbidity, most patients continue suffering decreased ventricular function and chronic heart failure.

Severe myocardial infarction conveys serious complications such as ventricular aneurysm, wall thinning and rupture with fatal consequences. Traditional aneurysm resection improves heart contractility by restoring the original oval structure of the left ventricle. This procedure is reserved only for patients with at least medium-sized ventricles because of volume consideration. Prognosis for these severe heart failure patients is three to six months of life despite CABG. Heart transplantation has been the gold standard for patients with end-stage heart failure, but donor heart non-availability and lifelong immunosuppression dictate its limited usage. It is urgent to explore new therapeutic measures to increase the quality of life and lifespan of these patients.

Chronic ischemia after AMI causes loss of cardiomyocytes, contractile filaments and ventricular contractility. Regeneration attempt by surviving cardiomyocytes consists of undergoing at most four mitotic divisions because the telomeric DNA repeats [1] in these terminally differentiated cells are minimal. Such attempt cannot regenerate enough cardiomyocytes to produce the necessary quantity of contractile filaments such as myosin, actin, troponin and tropomyosin to sustain normal heart contractility. The degenerative heart also transmits biochemical signals to recruit stem cells from the stroma and from the bone marrow to repair the muscle damage. Due to the significant increase in fibroblast growth factor release after infarction, much of the recruited stem cells differentiate to become fibroblasts instead of cardiomyocytes, forming scars and not contractile filaments.

The damaged myocardium needs replenishment of live, genetically normal, myogenic cells to deposit contractile filaments to regain heart function, preferably before fibroblast infiltration. This is when AMT has an advantage over all other cell types [2] [3]. Considering dystrophic and diabetic cardiomyopathies as hereditary, and ischemic cardiomyopathy as genetically predisposed, AMT was

designed to genetically repair hereditary degenerative cells and to replenish degenerated cells with live ones. In 1990, AMT was, and still is, the world's first human gene therapy and the world's first somatic cell therapy in the treatment of human muscle genetic disease [4].

Myoblasts are differentiated cells destined to become muscles. Unlike cardiomyocytes, myoblasts possess long telomere DNA subunits and can undergo 50 mitotic divisions without loss of myogenic capacity [2] [3] or developing into tumor [5]. The transition from animal experimentation [6] [7] into cardiac clinical trials was largely based on previous demonstration of significant safety and efficacy in the treatment of Duchenne muscular dystrophy in FDA approved Phase II and Phase III clinical trials using allogeneic myoblast allografts [8] [9] [10].

Law *et al.* reported in the year 2000 the pioneering study of implanting human myoblasts into the porcine hearts with endovascular catheter of the NOGA system [11]. The mechanisms by which allogeneic human myoblasts survived, developed and functioned with the use of cyclosporine immunosuppression were examined through open chest endomyocardial injections of cultured skeletal myoblasts into infarcted porcine myocardium. Three mechanisms were elucidated as proof-of-concept using genetic markers to label the nuclei of the donor myoblasts. Under the influence of hormones and slow contractile activity of the heart, donor myoblasts fused among themselves to form new cardiomyocytes, depositing contractile filaments to improve heart contractility. Others fused with the host cardiomyocytes through natural cell fusion, spontaneously transferring their nuclei into host cardiomyocytes to impart myogenic regeneration. Still others fused to form myotubes that eventually developed into immature skeletal myofibers containing satellite cells. New production of contractile filaments augmented heart contractility [2] [3] [12].

In 2004, Law *et al.* reported the world's first two cases of allogeneic myoblast transplantation for myocardial infarction [3] [12]. Results demonstrated that the left ventricular ejection fraction (LVEF), viability, motility, myocardial perfusion, and ventricular wall thickness were significantly improved [3] [12]. The end-diastolic and end-systolic blood volumes were increased without significant arrhythmia. At 12 months after implantation, LVEF was increased by about 40%, and perfusion capacity increased by 38% [3] [12]. More than 300 cases of autologous myoblast transplantation internationally have shown that it was safe and efficacious in treating ischemic cardiomyopathies and heart failure. Approximately 200 cases were injected after thoracotomy and 100 cases were injected with endovascular catheters [10] [12]. Further advance with myoblast clinical trials has been distracted for 17 years by Piero Anversa's misconduct in cardiac stem cell research in Harvard University until 10/14/2018.

2. Methods

2.1. Regulatory

This study was approved by the Ethics Committee of the Third Affiliated Hos-

pital of Xinxiang Medical College to obtain preliminary safety and efficacy information to justify future clinical trials of AMT. This study was registered in the Chinese Clinical Trial Registry numbered ChiCTR2000039590. All subjects signed Informed Consents for the trial.

2.2. Case Selection

A total of 10 heart failure subjects suffering severe myocardial infarction with ventricular aneurysm in the Cardiothoracic Surgery Department of the Third Affiliated Hospital of Xinxiang Medical College from February 2016 to March 2018 were selected. Subject profiles are shown in Table S1. Tables S1-S8 are placed in Supplementary Appendix.

2.3. Research Methods

Clinical evaluation was performed using a self-contrast method.

All patients were given CABG and conventional medication for symptomatic control of blood pressure, blood lipid, blood glucose, anticoagulation, coronary expansion, cardiac strengthening, and diuretics. Traditional aneurysm resection was not used in this study.

2.4. Manufacture of Allogeneic Human Myoblasts

Donors: Upon approval of the Institutional Review Board (IRB) of the Cell Therapy Institute and the signing of the Donor Informed Consents, muscle donors were admitted after meeting the Inclusion and Exclusion criteria. They were male volunteers between the ages of 16 and 36. They were certified by a physician as being in good health, having normal levels of aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LD) and tested negative for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis (RPR), and cytomegalovirus (CMV). They also received the following tests: Chem 24, CBC, and physical examination with normal results. Donors were excluded if they had any chronic or infectious diseases, and if were allergic to the local anesthetic Lidocaine.

Muscle Biopsy: About 2 grams of muscle were removed from the quadriceps muscle using an open biopsy technique under local anesthetic (Lidocaine patch) in a sterile field of a surgical suite of a hospital. The donor site was sutured and bandaged. No prophylactic antibiotic was used. The donor was discharged after recovery from the surgical procedure to be followed by his physician if infection occurred.

Preparation of Myoblasts: Biopsy specimen obtained was processed immediately using sterile techniques meeting CFDA approved GMP standards. Myoblasts were cultured in growth medium and incubated at 37˚C and in 7% CO_2 as previously described [13] [14]. Myoblasts were frozen at different stages so the time allotted for culturing could be coordinated with a scheduled transplant. The number of frozen cells and the number of samples were documented.

One test vial was reserved in liquid nitrogen for each biopsy. Random samples of the myoblasts were tested for their abilitytodivide, fuse, and formmyotubes [13]. Lotreleasetesting consisted of sterility, endotoxin, mycoplasma, and testing for myoblast identity, purity, potency, viability, and cell count on a pooled sample prior to transplant meeting quality control standards [13] [14] [15]. A retain sample of myoblasts was reserved from each transplant.

2.5. Immunosuppression

Each subject took daily oral doses of 5 mg/kg body weight of cyclosporine for three weeks with weaning using half doses after the second week to suppress rejection of the allografts. Since myoblast fusion completed within one week after transplantation [8] [16], and since myotubes and mature myofibers did not express major histocompatibility complex class 1 (MHC-1) surface antigens [8] it was not necessary to administer lifelong immunosuppression as in heart transplants [10].

2.6. Myoblast Transplantation

Following distal anastomosis of the coronary artery bypass surgery, transplantation of myoblasts was performed on the third day of cyclosporine administration. Approximately 1 billion myoblasts (at 108 cells /mL) were injected with 20 injections placed 1 cm apart along the inside border of the infarction. For each injection, about 50 million myoblasts were carefully deposited in a centripetal diagonal track (<5 mm) as the needle was slowly withdrawn out of the inner border of the infracted myocardium.

2.7. Safety Indicators

Blood routine (including white blood cell count, neutrophil fraction, red blood cell count, hemoglobin, platelet count), liver and kidney functions (including albumin, total bilirubin, alanine aminotransferase, glutamate aminotransferase, urea nitrogen, creatinine), electrolytes, bleeding and clotting times were determined before, and at 6 months and at 2 years after AMT.

Intraoperative and postoperative adverse reactions including virus infection, malignant arrhythmia, and death events were carefully monitored.

2.8. Efficacy Indicators

The clinical symptoms, vital signs, imaging examination and blood laboratory examination results of the patients before and after the operation were compared, and the adverse reactions during and after the operation were observed.

Observation Methods

1) Subjective symptoms (such as chest tightness, shortness of breath, number of angina pectoris, sit-up breathing at night, urine output, appetite, 6-minute walk test) before and after the operation were continuously observed.

2) Postoperative ECG monitoring

After treatment, all subjects underwent multifunctional 48-hour-holter monitoring to determine if there were changes in ECG and vital signs. An electrocardiogram was performed again at 6 months and at 2 years postoperatively to observe if there was occurrence of arrhythmia.

3) Echocardiography and cardiac ECT

Patients underwent Echo and ECT examinations before, at 6 months and at 2 years postoperatively to determine the LVEF, cardiac output (CO), and left ventricular end diastolic dimension (LVDd).

2.9. Statistical Analyses

Data were analyzed with one-way analysis of variance (ANOVA) using SPSS19.0 software. $P < 0.05$ indicated significant statistical differences.

3. Results

The authors vouch for the accuracy and completeness of the data and analyses and the reporting of adverse events and for the fidelity of the study to the protocol.

3.1. Safety Assessment

Adverse Reaction Assessment

All ten subjects successfully underwent treatment without malignant arrhythmia, chills, fever, allergic reactions, vomiting, viral infection or other adverse reactions.

Throughout the course of the entire study, two subjects exhibited early postoperative occasional ventricular premature beats. Another two subjects demonstrated atrial premature beats. Considered related to electrolyte disorders and myocardial damage of the primary disease, they were converted to sinus after symptomatic treatment. No malignant arrhythmia and no deaths occurred, and there was no virus infection after treatment.

There were no statistically significant differences between the means (±SD) in the leukocyte count, neutrophil count, hemoglobin, red blood cell count, and platelet count before versus at 6 months, and at 2 years after treatment ($P > 0.05$) (**Table S2** in Supplementary Appendix).

There was no statistically significant difference between the mean levels (±SD) of total bilirubin, albumin, alanine aminotransferase, and aspartate amino transferase before versus at 6 months, and at 2 years after treatment ($P > 0.05$) (**Table S3** in Supplementary Appendix).

There was no statistically significant difference between the mean levels (±SD) of serum creatinine or urea nitrogen before versus at 6 months, and at 2 years after treatment ($P > 0.05$) (**Table S4**) in Supplementary Appendix).

There was no statistically significant difference in the mean levels (±SD) of serum K^+, Na^+, Cl^-, and Ca^{2+} before treatment versus at 6 months and at 2 years

after treatment (P > 0.05) (Table S5 in Supplementary Appendix).

There was no statistically significant difference in the mean levels (±SD) of prothrombin time, fibrinogen, and partial prothrombin time before versus after treatment (P > 0.05) (Table S6 in Supplementary Appendix).

3.2. Efficacy Assessment

3.2.1. Objective Evaluation

Cardiac ECT demonstrated significant improvement in myocardial perfusion and in viability of ventricular myocardium at 6 months and at 2 years after surgery (Figure 1). The mean LVEFs were increased by 20.1% at 6 months after treatment, and by 19.3% at 2 years after treatment as compared to the control mean before treatment. These mean increases were of statistical significance at P < 0.05 (Table S7, Figure 1, Figure 2(A)).

There was no significant difference (Table S7 in Supplementary Appendix) in the mean levels (±SD) of LVDd (Figure 2(B)) and CO (Figure 2(C)) before versus after treatment (P > 0.05). Although the mean difference in CO before versus after treatment was of statistical insignificance, the postoperative means were all higher than before treatment. Consistently, the mean postoperative lengths of LVDd were lower than that before treatment.

MR imaging showed no significant difference in the mean levels (±SD) of ventricular aneurysm area and wall thickness before versus after treatment (P > 0.05) (Table S8 in Supplementary Appendix) though individual variations did demonstrate significant increases in wall thickness (Figure 3).

3.2.2. Subjective Evaluation

Postoperative clinical improvement included significantly fewer episodes of

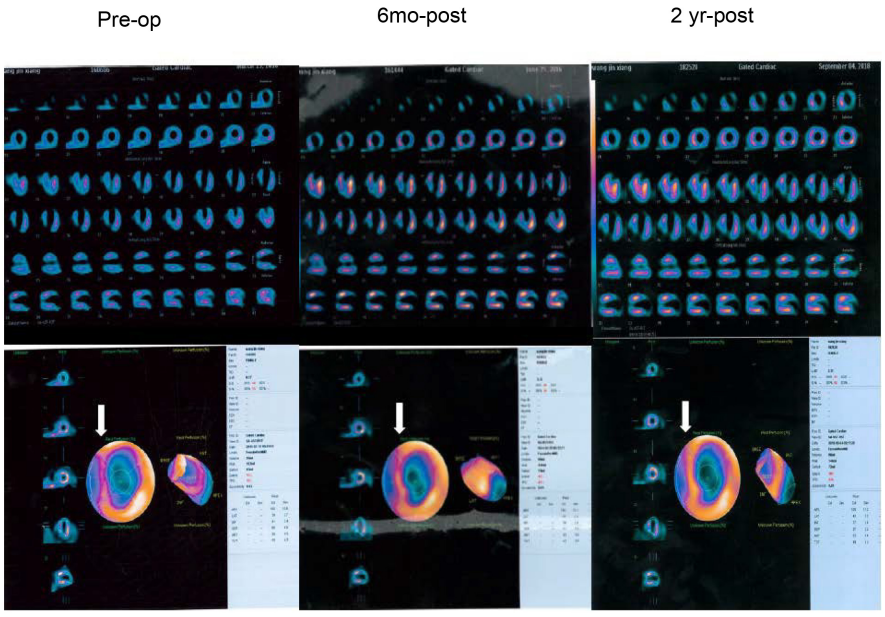

Figure 1. Representative ECT indicated significant increase in viability (Arrow) of ventricular myocardium at 6 months and at 2 years after surgery.

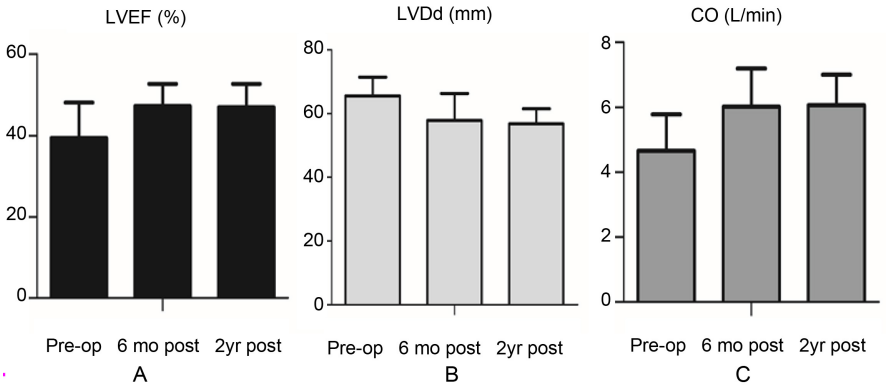

Figure 2. (A) LVEF showed significant increases at 6 months and at 2 years after treatment. There was no statistically significant difference in mean LVDd (B) and mean CO (C) before versus after treatment.

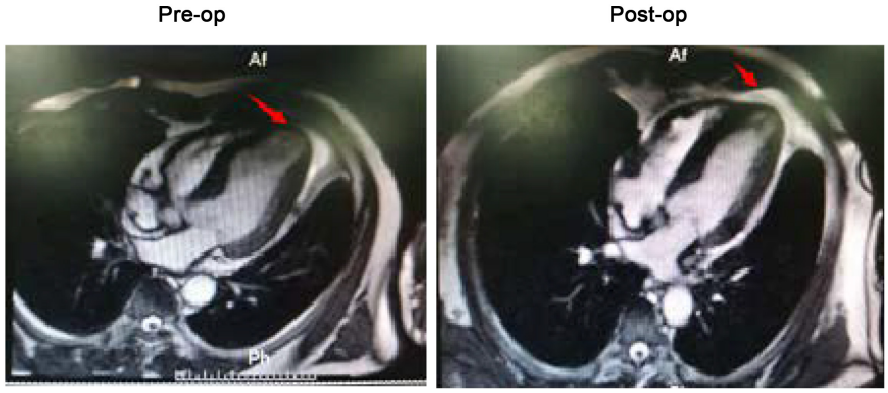

Figure 3. MRI indicated the increase in wall thickness after treatment (red arrows).

angina pectoris, chest tightness and shortness of breath after activity, nighttime sit-up breathing, and increase in appetite and urine output. The 6MWT distance increased steadily.

The results depicted essentially derived from allogeneic myoblast culture and transplantation (**Figure 4**), in addition to CABG and cyclosporine immunosuppression.

4. Discussion

For the first time, AMT in adjunct use with CABG and cyclosporine, was demonstrated to be safe and efficacious in sustaining the lifespan of end-stage heart failure patients, who suffered myocardial infarction with ventricular aneurysm, for up to 2 years, with significant improvement in LVEF (**Table S7**, **Figure 1**, **Figure 2(A)**), NYHA cardiac function (**Central Illustration 1**, **Figure 5**) and quality of life. It was the original design to define a treatment modality that could 100% sustain the survival of the severe heart failure patients having no more than 6-months of life expectance, and such design was demonstrated to be successful. The statistically significant increases in the mean LVEF by 20.1% at 6 months after treatment, and by 19.3% at 2 years after treatment compared

Figure 4. Schematic diagram of myoblast transplantation. (A) Myoblast culture of 20 roller bottles produced approximately 10 billion cells; (B) Myoblasts were produced under GMP condition; (C) Myoblasts of >90% purity according to desmin immunostain; (D) Approximately 10 billion myoblasts were injected into the myocardial infarcted ventricle.

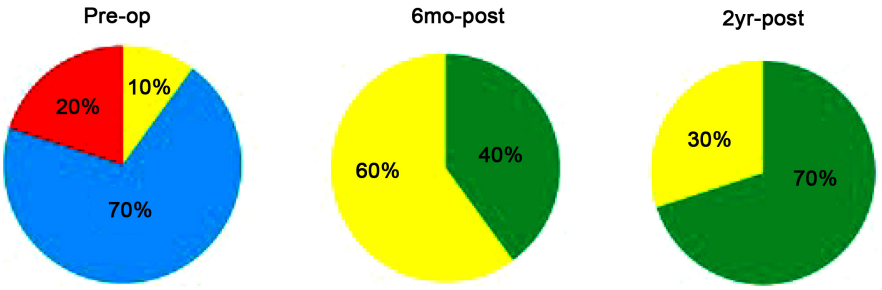

Figure 5. NYHA cardiac function improved by 2 grades at 2 years postoperatively. Green indicates Grade I, yellow II, blue III, and red IV.

favorably to those reported by all other studies using autologous myoblasts 17 - 33. As the trend of improvement indicated, statistically significant difference in LVDd and CO before versus after treatment would have become apparent if more subjects were enrolled.

The ultimate demonstration of AMT efficacy by itself should be from randomized, double-blinded studies involving more subjects of dystrophic cardiomyopathies, diabetic cardiomyopathy, or dilated cardiomyopathies that would not necessitate CABG.

This study showed that white blood cell count, neutrophil count, total bilirubin, alanine aminotransferase, aspartate aminotransferase, total red blood cells, hemoglobin, platelets, total protein, renal function, fibrinogen, prothrombin

time, and partial prothrombin time before versus after treatment of myoblasts were not different ($P > 0.05$). On repeated follow-ups after the operation, there were no deaths, malignant arrhythmias, or viral infections. The results demonstrated that allogeneic myoblast transplantation had no significant impact on various important indicators of the human body, and there was no serious adverse reaction. Although occasional ventricular premature beats occurred in four subjects after CABG, all symptomatic treatments were traced to the sinus and were related to electrolyte disturbance, myocardial damage and the primary disease. None of the subjects experienced malignant arrhythmia or ventricular tachycardia.

AMT represented the earliest human cell therapy for heart disease [11]. Without significant knowledge of myoblast manufacture, quality control and cell transplant techniques, many clinicians rushed into clinical studies. Menasche, *et al.* first reported feasibility and safety data on ischemic heart failure patients, implanting 650 million to one billion autologous impure myoblast cells with overly large number of injections into the infarctions during CABG [17] [18]. Follow-up studies showed that patients had significantly improved left ventricular function and NYHA function level, decreased ventricular remodeling, and increased myocardial tolerance confirmed by histology. However, 4 patients had delayed episodes of sustained left ventricular tachycardia [18]. During this period, similar reports continued to appear around the world with mixed results.

In most of the reports, myoblast purity was determined using CD56+, an antibody that reacts with fibroblasts, neurons, and myoblasts indiscriminately. A common pitfall of myoblast culture is fibroblast contamination. Since myoblast doubling time is about 21 hours and fibroblast doubling time is about 15 hours, fibroblast growth often overtakes the myoblast culture [13] [14]. Without published documentation of quality controls, authors of these studies were implanting very impure myoblasts. Fibroblasts produce scars but not contractile filaments. These scars created numerous barriers to electric coupling and rhythmic synchronization of ventricular contraction. This pitfall, together with the physical trauma induced by overly large number of injections, were largely responsible for the malignant arrhythmia and tachycardia reported in some early patients, not to mention the gross GMP non-compliance in quality control of myoblast identity, viability, purity, quantity, potency, no mycoplasma, no endotoxins and no bacteria [19]-[33]. The latter explained cell death of up to 90% that affected the safety and efficacy of myoblast transplantation [34]. Clinical studies confirmed that CABG with transplantation of impure and non-viable myoblasts produced ventricular tachycardia [26]. The intercalated disc, the basic unit for the transmission and synchronization of electrical activity and mechanical function between adjacent cardiac fibers were not found, and its absence constituted a risk of malignant arrhythmia after transplantation with substandard myoblasts [35]. Therefore, the safety and efficacy of myoblast transplantation depend largely on high quality control of myoblast production and the technique of implantation.

Heartbeat is myogenic in origin and is initiated by pacemaker activity in the sinoatrial node. As depolarization sweeps through the atrioventricular node, the depolarization excites the Purkinje fibers of the bundle of His, which, in turn, signals the ventricles to contract rhythmically. Heart function would be impaired if the rhythmic action potentials do not synchronize the fiber contractions. In the regenerative heart with AMT, excitation of the newly formed and heterokaryotic cardiomyocytes [2] [3] [12] remained unchanged because there was little change in gap junctions for current flow. However, where new skeletal myofiber [2] [3] [12] were formed, presumably at the inner border of the infarction, such heterogenicity might create aberrant electric activities such as arrhythmia, especially when earlier studies reported absence of gap junction protein connexin 43, a marker protein responsible for electrical coupling between cardiomyocytes [36].

Conceptually, myoblast transplantation should not cause arrhythmia if the well-researched standard operation procedures were followed. The thresholds of excitation for cardiac and skeletal myofibers are similar, *i.e.* between +40 mV to +50 mV depolarization. Whereas the cardiomyocyte action potential is triggered with an increase in Ca^{2+} conductance into the cell, the skeletal myofiber action potential is triggered with an increase of Na^{+} conductance. As Ca^{2+} has a greater ionic size than Na^{+} and thus lower ionic mobility, the action potential of cardiomyocytes has a longer duration (~250 ms) than that of skeletal myofiber (~1.5 ms). This duration difference is advantageous because the same myocardial depolarization can simultaneously and synchronously stimulate the cardiac and skeletal myofibers through direct excitation contraction coupling. Since the action potentials of skeletal myofibers are of short duration, they will merge into the longer compound action potential of the heart. The skeletal myofibers will cease to fire and stop contracting once Cl^{-} efflux hyperpolarization of the myocardium reaches approximately +40 mV. Since 90% of the transplanted myoblasts developed to become cardiomyocytes or heterokaryotic cardiomyocytes [2] [3] [12], and only a small amount of skeletal myofibers were formed, the electrophysiologic treatise explains why none of the subjects in the current study experienced malignant arrhythmia from the myoblast transplantation.

Abraham *et al.* [37] demonstrated in vitro that connexin 43 was expressed when exogenous connexin 43 gene was transferred into myoblasts. Electrophysiological studies had also found that there was a synchronized instantaneous calcium current between skeletal muscle myotubes and adjacent cardiomyocytes, further confirming that after transplantation, cardiomyocytes, heterokaryotic cardiomyocytes and skeletal myotubes were simultaneously activated through the same excitation-contraction coupling. Simultaneous contraction could not only promote the formation of gap junctions, but also effectively reduced the incidence of arrhythmia. In addition, a sodium ionic current was detected [38], confirming that some myoblasts developed to become myotubes and immature myofiber [2] [3] [12]. Skeletal myofibers are known to adapt to the frequency of electric excitation to which they are subjected. Under the influence of hormones and slow contractile activity of the heart, these immature skeletal myofibers de-

veloped characteristics of cardiomyocytes.

The regenerative heart with myoblasts was endowed with a greater number of myogenic cells capable of mitosis and was prepared to regenerate upon injury. These cells produced more contractile filaments to augment heart contractility. The latter is fundamental to the quality of life and the lifespan of patients suffering various forms of cardiovascular diseases. Being pluripotent, embryonic or adult stem cells exhibit uncontrolled differentiation into various lineages to produce bone, cartilage, fat, connective tissue, skeletal and heart muscles. Until scientists can accurately define the specific transcriptional factors and pathways to guide stem cell differentiation into adequate quantity of cardiomyocytes, the use of stem cell injection into the human heart would have a risk-benefit ratio much higher than the use of myoblasts.

Myoblasts are differentiated cells destined to become muscles. Further studies are necessary to better define the efficacy of myoblast transplantation itself, preferably through transplanting male donor myoblasts into female subjects, and using the Y-chromosome to track the development of implanted allogeneic myoblasts in the host. Position papers in 2017 highlighted numerous developing cell therapies for severe heart failure with neither governmental approval nor endorsement [39] [40]. Heart transplant has remained the generally accepted treatment for end-stage patients. With an estimate of over 50 million heart failure patients worldwide, only a few thousand donor hearts were available for transplants last year. Patients who survive heart transplants need to be immunosuppressed for life with not only compromised quality of life but also constant life-threat of COVID-19.

5. Conclusion

This study demonstrated survival and engraftment of allogeneic myoblasts in patients with ischemic cardiomyopathy. The improvement of heart function and the quality of life of patients was the result of a combination of CABG and cell therapy. A large randomized clinical trial will be required to demonstrate the efficacy and safety of AMT.

6. Perspectives

6.1. Competency in Medical Knowledge

AMT, cyclosporine immunosuppression and CABG were used to treat end-stage HF subjects without hope of obtaining a heart transplant.

6.2. Competency in Patient Care

AMT is much less invasive than a heart transplant. The regenerative heart with myoblast allograft is the patient's very own and requires only 3 weeks of immunosuppression.

6.3. Translational Outlook

At a small fraction of the cost of a heart transplant, the regenerative heart with

allogeneic myoblasts promises lower healthcare spending if proven safe and efficacious.

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Authors' Contribution

Prof. Li, Prof. Law and Dr. Wang contributed equally and are co-first authors. W.L., H.Q., F.W. performed surgery; H.G., B.Z. provided postoperative intensive care; K.W., L.L., F.L. collected data and conducted statistical analyses; Q.S. provided patient coordination; H.F. conducted extracorporeal circulation, J.L., Y.L., Y.Y. conducted postoperative follow-up; P.L. supervised myoblast manufacture; D.C. provided myoblast quality control; D.M.L provided project coordination; P.K.L. designed project and analyzed data, provided free licenses and myoblasts, and wrote manuscript with K.W.

All authors read and approved the final manuscript and declared no conflicts of interest regarding the publication of this manuscript.

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Supplementary Appendix

Table S1. General information of subjects.

	Gender	Age	Combined disease	Infarct area	Abnormal movement (Y/N)	Bypass vessels
Wang	F	53	History of cerebral hemorrhage, hypertension	Anterior and interval wall	N	LAD, LCX, D1
Huang	M	31	None	Anterior wall near apex	N	LAD, LCX
Cui	M	72	Membranous nephropathy, diabetes	Anterior, interval and rear wall.	N	LAD, LCX
Ren	M	62	Hypertension	Anterior wall near apex	N	LAD, LCX
Song	M	60	Hypertension, diabetes	Anterior, interval and inferior wall.	N	LAD, LCX, D1
Zhou	M	53	None	Anterior wall near apex	N	LAD
Wang	M	68	None	Anterior wall near apex	N	LAD
Che	M	60	Hypertension	Anterior wall near apex	N	LAD, LCX, LCA
Du	M	60	None	Anterior wall near apex	N	LAD, LCX
Zhang	M	64	Hypertension, diabetes, history of cerebral hemorrhage	Anterior wall near apex	Y	LAD, LCX

Table S2. Blood cell test results before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
Leukocyte count	7.59 ± 3.19	8.29 ± 2.45	8.18 ± 1.95	0.808
Neutrophil count	3.25 ± 0.88	3.35 ± 0.55	3.24 ± 0.75	0.934
Hemoglobin	147 ± 9.94	144.8 ± 9.94	142.8 ± 9.42	0.635
Red-cell count	4.41 ± 0.63	3.95 ± 0.60	4.04 ± 0.77	0.279
Platelet count	213.4 ± 48.95	245.60 ± 52.15	236.10 ± 40.36	0.312

Table S3. Liver function indexes before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
Total bilirubin (μmol/L)	18.02 ± 10.6	20.31 ± 10.17	15.46 ± 5.11	0.491
Albumin (g/L)	41.98 ± 4.46	42.76 ± 3.74	44.25 ± 2.52	0.138
Alanine aminotransferase (U/L)	43.80 ± 34.77	29.80 ± 18.67	24.20 ± 5.98	0.166
Aspartate amino transferase (U/L)	30.25 ± 7.29	30.5 ± 7.8	26.13 ± 7.74	0.161

Table S4. Renal function indexes before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
Serum creatinine (μmol/L)	76.90 ± 16.91	112.40 ± 81.32	82.80 ± 14.77	0.236
Urea nitrogen (g/L)	4.11 ± 1.05	4.78 ± 0.98	4.03 ± 1.05	0.776

Table S5. Electrolyte levels before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
K^+	3.98 ± 0.35	4.08 ± 0.42	4.01 ± 0.35	0.831
Na^+	140.6 ± 2.98	142.1 ± 3.31	142.1 ± 2.80	0.456
Cl^-	102.30 ± 2.83	103.3 ± 3.40	103.9 ± 5.52	0.680
Ca^{2+}	2.17 ± 0.09	2.29 ± 0.10	2.26 ± 0.13	0.075

Table S6. Blood coagulation indexes before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
Prothrombin time (s)	13.18 ± 0.50	13.30 ± 0.54	4.01 ± 0.35	0.951
Fibrinogen (g/L)	3.13 ± 0.17	3.26 ± 0.17	2.88 ± 0.20	0.341
Activated partial thromboplastin time (s)	29.55 ± 1.13	30.94 ± 0.98	32.63 ± 1.22	0.169

Table S7. Cardiac function before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
LVEF	39.3 ± 8.76	47.2 ± 5.44	46.9 ± 5.69	0.029
LVDd	60.3 ± 9.86	57.3 ± 8.84	54.8 ± 7.24	0.381
CO	5.18 ± 1.12	6.02 ± 1.18	6.07 ± 0.94	0.351

Table S8. Ventricular aneurysm area and thinnest wall thickness before versus after treatment.

	Pre-op	6 mo-post	2 yr-post	P
Area, mm^2	460.1 ± 143.14	394 ± 150.93	422.7 ± 426.31	0.864
Thickness, mm	2.21 ± 0.55	2.32 ± 0.51	2.49 ± 0.70	0.565

Central Illustration 1

NYHA grading before versus after treatment.

	Gender	Age	Pre-op	6 mo-post	2 yr-post
Wang	F	53	III	II	II
Huang	M	31	III	I	I
Cui	M	72	III	II	I
Ren	M	62	III	I	I
Song	M	60	IV	II	II
Zhou	M	53	III	II	I
Wang	M	68	II	I	I
Che	M	60	III	I	I
Du	M	60	IV	II	II
Zhang	M	64	III	II	I