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Effection of myocardial cell/collagen compound on ventricular electrophysiology in rats with myocardial infarction

Q. RUPING[1], B. KUKEN[2], Y. HUANG[3], J. SUN[1], A. AZHATI[1]

[1]Heart Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China
[2]Cardiovascular Department, The Second Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China
[3]Department of Electrocardiogram, Huadong Hospital, Shanghai, China

Qian Ruping and *Bannu Kuken* contributed equally to this work

Abstract. – OBJECTIVE: Microelectrode array (MEA) technique could synchronous record of electrical activity for many cardiac cells to detect the interval difference between excitation produced by the same pacemaker cell or different pacemaker cell to different electrodes. In this experiment study, the MEA technique was applied to study cardiac cells/collagen complex affected the electrophysiological properties in rat ventricular muscle and expound the discovery that cardiac cells/collagen complex can repair rat heart functions.

MATERIALS AND METHODS: Thirty SD rats were evenly divided into three groups: control group, myocardial infarction group and transplantation group. The control group underwent thoracotomy without coronary artery ligation. The transplantation group was transplanted with tissues compounded with myocardial cells and collagen materials after the model was established. The amplitude and the activation-recovery interval of myocardium were recorded.

RESULTS: The field amplitude of the ventricular myocardium in the infarction zone, opposite zone and border zone in the transplantation group increased compared with the MI group. The activation-recovery interval in the MI group, as well as the infarction zone and infarcted border zone in the transplantation group, was variously prolonged ($p < 0.05$).

CONCLUSIONS: Myocardial cell/collagen compound could replace infarction tissues and improve electrophysiological characteristics in myocardial infarction rat, partly repair function of the heart.

Key Words:
Engineered cardiac tissue, Myocardial infarction, Microelectrode arrays, Myocardial cell/collagen compound.

Introduction

Ischemic heart disease has become the main cause of death after acute myocardial infarction. Myocardial cells reduction will lead to severe heart failure and complications. Tissue engineering was a rapidly developing subject, its goal was a combination with cells, biomaterials and biological activity of molecules to manufacture, repair and replace tissue and organ repair myocardial, which provided a possibility for repairing damaged myocardium. Cardiac tissue engineering focuses on tissue engineering. During the culture process *in vitro*, tissue environment *in vivo* is simulated to establish 3D complexes that are made up of cells and biomaterials, this environment is the place for cell growth and metabolism and the basis for cell differentiation to form new tissues and organs with new shapes and functions[1-4]. Microelectrode array (MEA) is a new technique which is used to record tissue action potential. This technique can directly reflect electrophysiological activities at the place where electrodes fire signals[5-8]. This experiment measures the effects of transplanted cardiac cells/collagen complex on the electrophysiological properties of the ventricular muscle with MI in rats using the MEA technique.

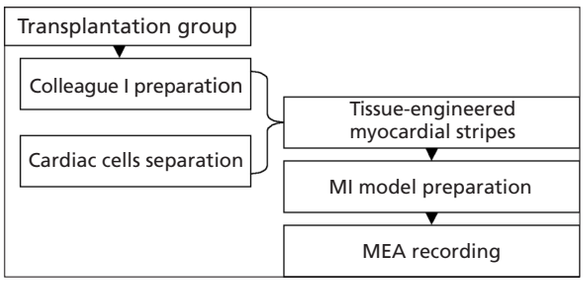
Materials and Methods

Animals and Grouping

Thirty female adult Sprague Dawley (SD) rats, which weigh 220 g to 250 g, were randomly divided into three groups: the control group, myocardial infarction (MI) group and transplantation group (n=10, each group). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Animal Experimentation and Ethics Committee of Xinjiang Medical University.

Corresponding Author: Adila Azhati, MD; e-mail: coronary_1_lab@126.com

Transplantation group protocol.



Colleague I Preparation

The rat tails were cut down from the end of SD rat tails and were immersed in 75% ethyl alcohol for 30 min. The tail tendon was torn off and cut down under aseptic conditions. Up to 1.5 g tail-tendon fragments were immersed in 150 ml of 0.1% acetic acid, which were placed in a refrigerator at 4°C and stirred using a magnetic stirrer. After 48 h, the supernatant liquid was collected by centrifugation to obtain colleague solution. The prepared colleague solution was measured to contain 1.9 g/l colleague by electronic balance.

Cardiac Cells Separation

100 newborn SD rats were immersed and killed in 75% ethyl alcohol. Ventricular muscle tissues were placed in a sterilised condition, cut into 1 mm^3 pieces, placed in wells and washed twice with Dulbecco's modified Eagle medium (DMEM). The tissues were then placed in 0.1% pancreatin in a 37°C water bath for 8 min digestion. The tissues were gently blown and beaten for supernatant liquid absorption, and serum was added to discontinue digestion. The above operations were repeated to treat the remaining tissues until all tissues have been digested. Stainless sifter of 200 meshes was used to filter and collect the supernatant liquid; this procedure was followed by centrifugation of collected cells and the addition of 10% serum DMEM (Boshide Biological Company, Wuhan City, Hubei Province, China) culture solution. Afterwards, the tissues were crushed evenly and subsequently placed in an incubator at 5% CO_2 and 37 °C for 1 h cultivation to remove the fibroblast. The cells that did not attach well to the wall were mainly cardiac cells.

Preparation of Tissue-Engineered Myocardial Stripes

1.9 g/L liquid colleague I was mixed with 2 × DMEM proportionally, and the mixture was neutralised by 0.1 mol/L NaOH. Subsequently, 10% Matrigel basement membrane matrix was added. Afterwards, the separated rat cardiac cells were mixed with the above mixture, resulting in a 2×10^{10} L^{-1} concentration. This mixture was added to a ring tank and placed in an incubator at 5% CO_2 and 37°C. After 1 h, the mixture was added to the culture solution. Cardiac cells/colleague mixture solution can be solidified into cardiac cells/collagen complex of stripe shapes. All 15 stripes of cardiac cells were constructed; these stripes were cultivated inside a tank for 7 d and removed from the compound bands. The stripes entangled two parallel round metal bars in a static stretching device, and the distance between two metal bars was adjusted so that the band can be statically stretched by 10%. The stripes were further cultivated *in vitro* for another 7 d[9].

Control Rats Operation

The rat chests were opened but their coronary artery was not ligated. The rats were fed with routine fodder for 4 weeks after the modelling procedure was finished. This method was followed by relevant electrophysiological detection.

MI Model Preparation

SD rats were anaesthetized by an intravenous drip of 10% chloral hydrate solution (3 ml/kg). The rats were fastened to the experiment bench and their chests were cut open along the central line of the sternum. The pericardium was cut, fully exposing the heart, the anterior descending branches were ligated by 5/0 sterile suture at 1/2 way between the left auricle and cardiac apex. Electrocardiogram I, II and aVL leading ST segment elevation 0. 1 mV signify successful operation (Figure 1). The rats were fed for 4 weeks inside the cages after the incisions were sutured.

Transplantation of Myocardial Cell/Collagen Material Compound

Myocardial infarction rats chests were reopened, exposed the ante theca of the ventriculus sinister and separating the adhesion between the pericardium and MI zone. Four corners of tissue-engineered myocardium stripes were subsequently sutured by silk thread 1 to the MI zone to establish close contact. The rats were fed with routine fodder for 4 weeks after the modelling procedure was finished.

Field Potential Duration Recording

The rats underwent abdominal anaesthesia using chloral hydrate (3 ml/kg), and chests were

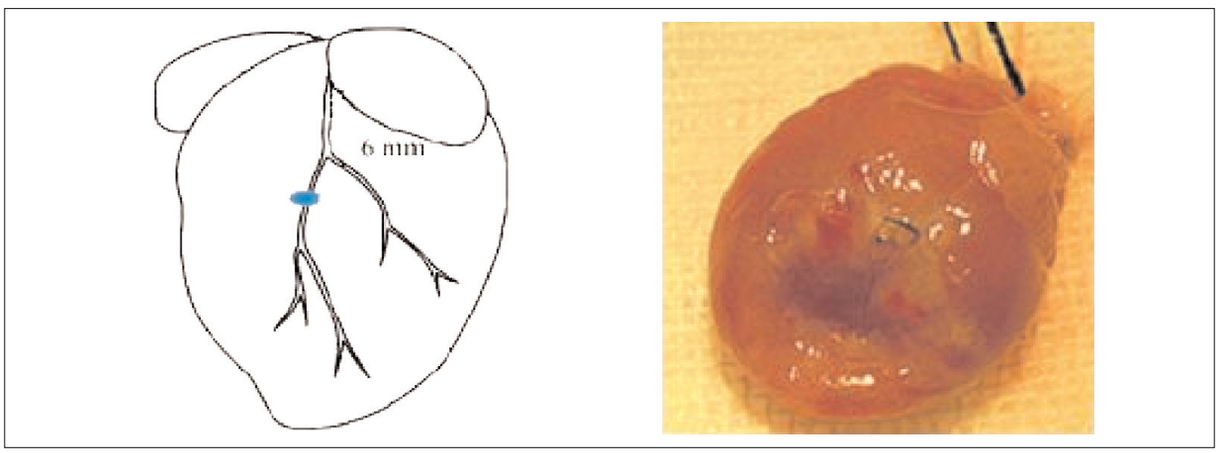


Figure 1. Low ligation schematic diagram of the left coronary artery.

opened to remove the hearts. Langendorff profusion heart was used at a 1.5 mL/min speed to perfuse the hearts; the hearts were placed in MEA perfusable electrode wells whose temperatures were controlled at 37 °C in CO_2 temperature control device. Some areas of field potential morphology of the left ventricle were recorded.

Statistical Analysis

The software SPSS16.0 (SPSS Inc., Chicago, IL, USA) was used to treat the data. The metering data will be represented by the means ± standard deviation (), and the comparison between groups was obtained using the *t*-test for the two independent samples in complete random design. The comparison of the electrophysiological index in different zones of ventricular muscle was detected by ANOVA. $p < 0.05$ was considered statistically significant.

Results

Successfully Making MI Model

Electrocardiogram displayed I, II and aVL leading ST segment elevation 0.1 mV. The results signified successfully making MI model. The rat hearts were dissected after making model 4 weeks, and identified pale colour in infarction zone with distinct demarcation with non-infarction zone in the MI group. Tissue-engineered myocardial strips covered the infarction zone and some myocardial strips and MI were combined as a whole in the transplantation group.

Ventricular Field Potential Morphology and activation-Recovery Time in the Control Group

Extreme wave shape and wave forms from normal rat ventricles were observed in three dimensions, which showed RS and rSR shapes. The amplitude of the main wave in the left ventricle was 485 ± 21.21 mV. The activation time for the front wall was 235 ± 7.07 ms, 241.7 ± 7.64 ms for the back wall and 235 ± 5 ms for the free wall (Figure 2).

Ventricular Field Potential Morphology and Activation-Recovery Time in Myocardial Infarction Group and Transplantation Group

Extreme waves showed large differences in different parts of MI rats. The wave forms of the

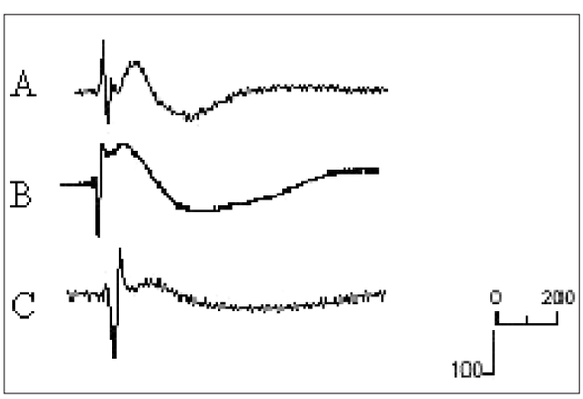


Figure 2. Microelectrode arrays record ventricular field potential morphology in the control group. ***A,*** Left ventricular anterior wall; ***B,*** Left ventricular posterior wall; ***C,*** Left ventricular free wall.

ventricular front wall in the MI group were mainly in QR or qR form, R wave was round and blunt with 135.00 ± 7.07 mV amplitude, whereas wave forms from the front wall and free wall of the ventricle were mainly in R wave form with 175.00 ± 15.00 and 92.50 ± 15.54 mV amplitude. Depolarization and repolarization time for ventricular muscle in the MI zone were extended; activation-recovery time reached 390.00 ± 26.77 ms. Activation-recovery time was extended in different ventricular muscle parts of the MI group, as well as the border of the infarction zone. However, the activation-recovery time in the border zone was less than the time in the former ($p < 0.05$). The wave form of the ventricular front wall in the transplantation group was mainly in QR or qR wave form. The wave form of the ventricular back wall (opposite to MI) and free wall (border zone of infarction parts) was mainly in Rs or R wave form (Figure 3).

The amplitude of main waves in the infarction zone, opposite zone and border zone in the transplantation group 3 increased compared with that in the MI group ($p < 0.05$). The activation-recovery time for the infarction zone, opposite zone and border zone of the transplantation group shortened compared with that of the MI group ($p < 0.05$). In different parts of ventricular tissue, the activation-recovery time for the infarction zone and infarction border zone of the transplantation group was prolonged; however, the time was less than that in the MI group ($p < 0.05$) (Figure 4).

Discussion

The finding indicates that MI leads to systolic and diastolic heart function damage in rats. The field potential amplitude of the transplantation group decreased but was still higher than MI group. Therefore, cardiac cell/collagen compound could improve the left ventricular systolic and diastolic heart function. The experimental results showed that MI had effects on activation-recovery time of different field potentials in ventricular tissue. The activation-recovery time in the infarction zone and the infarction border zone was prolonged. The activation-recovery time for the back wall was apparently shortened. All back walls of ventricular tissue were composed of normal cardiac tissue parts whose activation-recovery interval was normal. The activation-recovery interval in MI group was longer than the control group. Cardiac cells/collagen compound might affect the electrophysiological change by repairing the structural functions of the MI infarction zone. This process leads to shortening of the intervals or depolarisation and repolarisation of cardiac muscles.

Clinical practice has proved that myocardial would not be recycled after subjecting to irreversible damage and gradually replaced by fibrous tissue, eventually develop heart failure. Surgical transplantation could therapy myocardial structure or myocardial function loss associated myocardial disease. Because the heart transplantation donor and graft rejection are restricted,

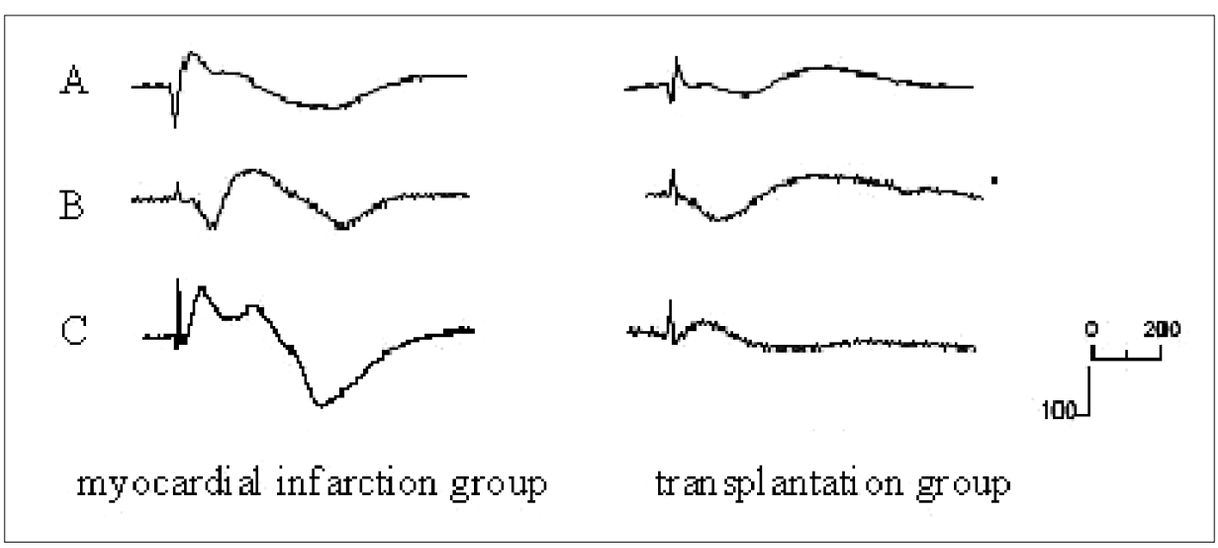


Figure 3. Microelectrode arrays record ventricular field potential morphology in model group and transplantation group. ***A,*** Infarction zone; ***B,*** Opposite zone; ***C,*** Border zone.

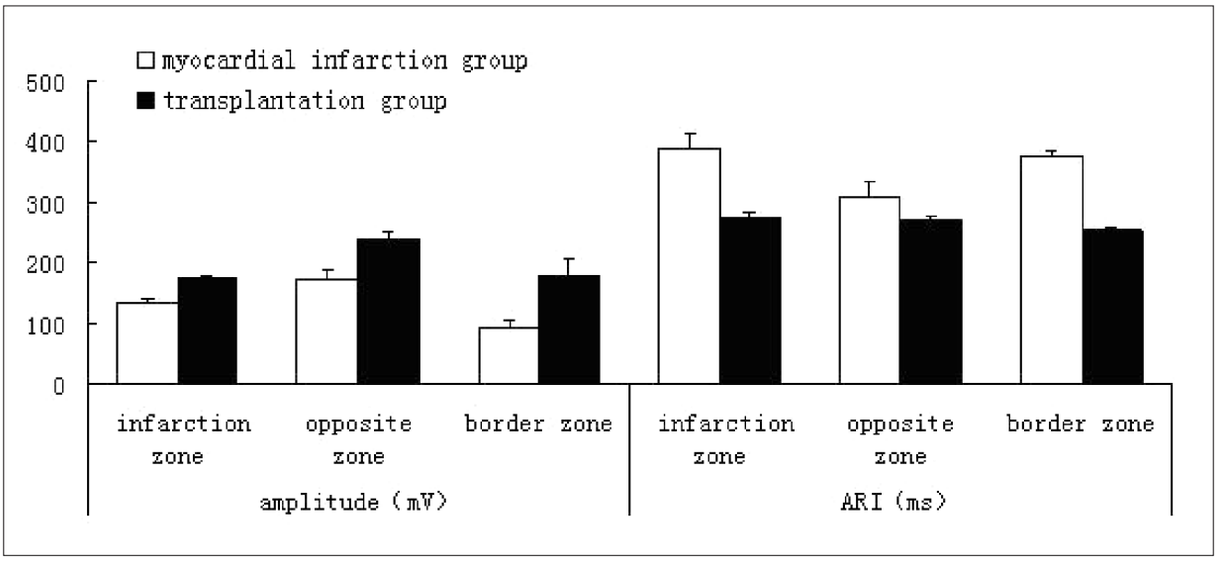


Figure 4. Comparison of amplitude and ARI in MI group and transplant group. Note: compared with myocardial infarction group, $*p < 0.05$.

which limited application of surgical transplantation. The key technology of tissue engineering was the cells cultured *in vitro*, the simulated environment *in vivo* tissues, and constructed a three-dimensional space of complex of cells and biomaterials. In cardiac tissue engineering research, Eschenhagen et al[10] transplanted chicken embryonic cardiac cells into collagen gels; this procedure formed contractile cardiac muscle network structure *in vitro*. Leor et al[11] transplanted cardiac cells from the foetus into a porous gelatine scaffold. The compounds were transplanted into the MI scarred zone. These procedures resulted in the formation of extensive neovascularisation in the transplanted graft, and no left ventricular dilation, cardiac insufficiency and other symptoms were noted.

Conclusions

MEA technique was used in this experiment to compare the left ventricular field potential amplitude in three groups. Higher amplitude resulted in better heart contraction and stretching. The field potential amplitude of the MI group was lower than the control group. Which indicated myocardial infarction to lead to damage of systolic and diastolic heart function and cardiac insufficiency. The amplitude of the field potential in transplantation group was higher than that MI group, which meant that cardiac cells/collagen compound could improve the systolic and diastolic heart function of the left ventricle.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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