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An Alternative Technique for Heart Lesion in the Rat: A Step to Fetal Heart Implantation for Cardiac Tissue Repair Running Title: A New Technique of Heart Lesion in Rat

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Abstract

Existing experimental models of cardiac lesion do not allow precise reproducible dimensions of injury with complete safeness of surrounding tissues and cannot answer the tremendous question for heart cell therapy: are the implanted cells unable to influence the heart healing process or do the conditions in the injured heart prevent their complete development and integration?

60 rats were used for elaborating an alternative technique of cardiac lesion.

The anterior apical area of the heart was cauterized with a «Cautery high temperature fine tip» to create an injury of controllable, precise and reproducible dimensions (8×8 mm² surface, 1 mm depth).

To evaluate functional and morphological characteristics of the lesion, electrocardiography, pulse oximetry, echocardiography and optic microscopy were performed at different times (from day 0 to 230) after the operation.

After technical adjustment a 100% survival of the last 15 operated animals was obtained. A sub epicardium "infarction" was documented: ECG mirror ST-modifications in 2 leads, stable ejection fraction significant decrease, necrotic alterations and fibrosis of the lesion area, with surrounding myocardium preserved.

The survival and the injury evolution suggest that the proposed technique could be used for studies concerning cardiac tissue repair.

Keywords: Electrocardiography; Heart disease; Cardiac surgery; Cardiac lesion imaging; Ventricles aneurysm

Introduction

Nowadays, because of the increasing of the prevalence and cost of cardiac insufficiency [1-6] and the difficulties to do more than slowing its natural evolution, the interest for cell therapy is rising. Many studies were provided in this direction, but it was reported that the injured heart remodeling was not always favorably influenced by stem cells or cultured cardiomyocytes implantation [7-11]. Such trials were performed either in infarct patients or in animals after coronary artery ligation. So the question remains: are the cells incompetent, unable to influence the heart healing process or do the conditions in the injured heart prevent the implant complete development and integration?

Conversely to stem cells cultures, ectopic implantation of fetal hearts in vivo has led to complete development of an adult-like functional organ [12,13]. But they have neither been implanted at the heart site nor tested on cardiac defect repairing yet.

So it seemed to be important to use an experimental model of cardiac lesion which could offer optimal conditions for the implant development.

Several experimental techniques of heart injury were elaborated for the study of cardiac infarction and its remodeling. Heart main artery obstruction was obtained by physical means: cold or heat injury, radiofrequencies [14,15], chemical methods: injections or application of chemicals on the myocardium [16-18] or surgical ones as vessel ligation [19]. Presently the most usual experimental model of creating a cardiac lesion remains the temporary or permanent ligation of a coronary artery [9-12,19-22] or other type of heart vessels occlusion [23]. The advantage of this model is its proximity with the injury caused by myocardial infarction in clinical practice. The disadvantages include the variability of the lesion extent due to the difficulty to obtain a precise location of the coronary artery (or one of its branches) ligation/occlusion and the variability of collateral vessel types [20-23]. Moreover, the myocardial ischemia around the infarction zone seems to be unfavorable for cells or tissue transplants development that explains the controversial results of such a procedure [7-11,20,23].

The purpose of this study is to provide an alternative technique of cardiac injury by creating a size and depth controllable myocardial lesion.

The benefits would be the reproducibility of the lesion, but also the opportunity to study the most favorable time to practice cardiomyoblasts (fetal heart) grafting.

Methods

Surgical procedures

The experiments were performed on 60 rats. They were displayed

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in 4 series: 1/ for testing the feasibility of the technique – 12 animals (Wistar and Fischer, males and females); 2/ for improvement and standardization of the technique, determination and correction of its complications – 18 animals (Wistar: 14males, body weight (BW) 387-434 g and 4 females, BW 240-280 g); 3/ for testing the reliability of the technique – 19 animals (only Wistar males, aged 9.1+/-1.7 months, BW 427+/-28 g, among which 4 died during the operation from narcosis or ventilation problems, and were excluded); 4/ control series including 11 intact animals (only Wistar, males, same age because siblings, and BW 414+/-33 g).

Anesthesia and analgesia were provided as follows: induction by Isoflurane (4,5% for 1 min/100g BW), main procedure by 2 intra-peritoneal injections of Natrium pentobarbitalum (Nembutal® - Ceva Santé animal- Brussels Belgium: 0.1 ml/100 g BW of a solution to 0.075 mg/dl) and of Buprenorphine hydrochloride (Temgesic® - Laboratoire Schering-Plough – Courbevoie France: 0.2 ml of a 0.05% solution). A subcutaneous injection of 0.2 ml (1%) solution of Atropine (atropine sulfate – Lavoisier- Paris-France) was performed at least 5 minutes before intubation to avoid the occurrence of a vagal shock. The tracheal intubation with a 14 G catheter was performed with the help of a laryngoscope (Mac 0 blade-Heine Germany). The anesthesia-ventilation machine (Intermed–Penton, Sigma Delta, UNO-Netherland-USA) was used during the surgical procedure at a rate of 60 breaths/minute with a tidal volume at 12 ml/kg and ventilation pressure of 0 to 20 milliBars.

After longitudinal sternotomy and hemostasis, the heart was exposed.

We used the «Cautery high temperature fine tip» (Bovie Medical Corporation-USA) to induce, by several drop-contacts without any pressure – less than 1 second each - of the tip (standard temperature of 1200°C) with the surface of the heart, a myocardial lesion at the level of the anterior apical zone of the heart (including mainly left ventricle but also parts of septum and right ventricle). This localization was chosen because it is easy for access, relatively safe, as far as the left ventricle thickness is no less than 2 mm at diastole, and because the vessels of this zone are terminal, preventing an unexpected extension of the damage through major coronary branches lesions. The surface and the depth of the lesion depended on the total time of the tip contact with the myocardium. It was important to leave the internal muscular layer intact (ensured by visual control) in order to avoid immediate or delayed perforation (Figure 1). The extension of the damage was controlled by histology performed immediately after operation and during the follow up (see below). The thoracic wall wound was then sutured layer by layer with classic separate stitches using Vicryl 2°° for sternum, 4°° for muscles and diaphragm (if necessary) and continuous suture 6°° for the skin.

During the operation and the early - first hours - follow-up, the impact of the lesion on the heart function was monitored by a "Pulse Oximeter" (Contec Medical systems Co, Ltd PRC – RP China; CMS-508 model) fixed to the hind left leg of the animal. Electrocardiogram (ECG – leads I, II and III), respiratory rate (RR) and rectal temperature were registered by a «Mouse Monitor» (Indus Instruments USA - UNO-Netherlands). ECG and RR were obtained from 4 needle electrodes subcutaneously inserted in the standard left - right axillar and groin sites, the rat being in supine position.

Later, daily observation of the animals was realized up to 8 months after the operation; the animal body weight (BW) was measured at days 2, 5, 7, 14 and, after the initial BW recovering, once a month.

Post operation investigations

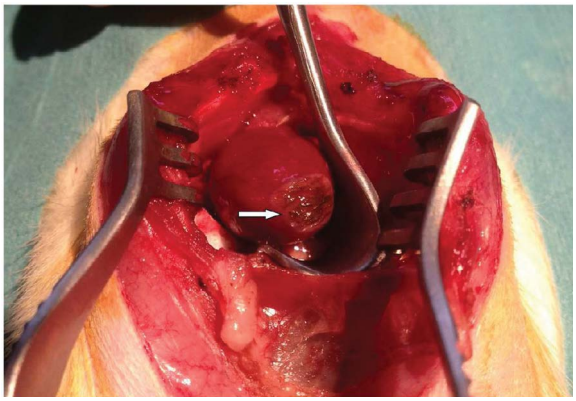

Figure 1: Per-operative macro photography of the myocardial lesion at the apex zone of the heart (arrow).

1.1.1. **Echocardiography:** At days 10+/-2, 30+/-5, and later at 1.5, and 4, 5, 6, 7, 8 months, the heart-operated animals were investigated using an ultrasound system IU22 (Philips-NL) with an ultrasonic probe (L17-5 MHz). 11 intact animals were also investigated and used as controls. Ejection fraction (EF) was determined by Simpson method. The left ventricle wall thickness (LVWT) was measured at the level of the lesion and in the intact area of the heart. To test the liability of the echocardiography data, in 2 intact animals the parameters were measured 5 times at a 1-week interval. To test the intra observer variability during each investigation, the EF and LVWT measures were repeated 3-4 times and M+/-SD values were calculated. The variability of the investigation results was determined as the quotient of standard deviation and means expressed in per cents of the mean (V=SD/M%). The reproducibility of the obtained results was determined as good if >90% (variability <10 %), acceptable if 80-90%.

In order to avoid pain during echocardiography investigation of recently operated animals anesthesia by intra peritoneal injection of Pentobarbital Sodium (Nembutal®: 0.1 ml/100 g BW of a solution to 0.075 mg/dl) was provided.

Histological control: In all series, after each animal death or euthanasia (anesthetic overdose), that is at days 0, 1, 2, 5, 7, 14, 21, 28, 35, 37, 42, 60 and at months 4, 6 and 7.5, collected biopsy material from heart, lungs and liver was fixed in neutral buffered formaldehyde 4% and embedded in paraffin. Three microns slices were stained by hematoxylin eosin and trichrom green of Masson for microscopic evaluation of the damage.

Statistics: The results are given as Mean +/- Standard Deviation (M+/-SD). Differences were tested by Student's test versus control series (td<t standard).

Results were considered significant for $p < \text{or} = 0.05$.

Besides, the statistic evaluation concerned not only the mean data of each series but also the differences between the results obtained at days 10 and 30 in the same animal (paired results).

Variability (V) and reproducibility (R) of the results were also calculated (V=SD / M %; R=100-V %).

Animal's management: Before, during and after the experimentation animals were managed according to the international rules of Bioethics and the experimental protocol was agreed by the local Ethic Committee (N°508N). The nutrition conditions were standardized all through the experiment with the use of "food for breeding" (AO4, "Safe"; France) and of fresh water as drink.

Results

The global results of our experiment are presented in Table 1. Their details are noted in Table 2.

The preliminary 12 experiments of series 1 have allowed determine the conditions for successful operations: 1/ appropriate assisted ventilation, 2/ cauterization by repeated short contacts (<1 s providing an alteration of about 1 mm^2/shot) of the cautery tip with the heart surface till the lesion demanded dimensions were reached.

The survival of 2 animals encouraged to continue improving the operative technique.

The next series 2 was devoted to the development of a standard, reproducible method: a necrotic lesion of 0.8 mm diameter area was visually checked, its depth was modulated by the duration of the cautery tip application on the surface of the heart. The survival was 50%. When this lesion was transmural, that is when the inner muscular layer of the ventricle was involved, it caused an immediate, uncontrollable and lethal bleeding (2 cases) or a late hemorrhage (1 case at day 37). When the injury only affected from 25 to 75% of the myocardium thickness, this was compatible with survival (9 cases - see Table 2 and Figure 1). Proper lesion was obtained with repeated short applications of the tip on the heart surface during less than 1 second each for a total duration no more than 50 seconds. Besides, the deep myocardial layers must be left "intact" (confirmed by macro and microscopic investigations).

Significant changes – QRS slight enlargement (up to 25 millisecond), ST alterations were already observed at the time of the lesion forming. Within 30-50 minutes after the operation significant elevation of ST in lead I and mirror lowing of ST segment in lead III (more than 30 millivolts relatively to basic line) of ECG have been recorded (Figure 2). After spontaneous ventilation recovery, blood oxygen saturation was decreased, compared to the situation before intubation (93 +/- 2% against 97 +/- 0.5%), despite increased respiratory rate (up to 100/min instead of 60-80/min).

The main complications in this series 2 were thoracic parietal bleeding (1 case), perforation (1 case) and severe cardiac or respiratory insufficiency (6 cases), causing early death of the animals within the first 4 hours after surgery. Among the 9 long-term survivors, we could observe one case of late thoracic bleeding (at day 17). The other animals survived without any evident complication except that their body weight did not increase as in control animals. The echocardiography, provided 52 days and 7 months after the operation, has shown some stiffness of the anterior apical area of the heart in the zone where the lesion was performed.

Animal number	Death at Day 0-1	Early lethal complication	Complication number	Survival	Late complication
Series 1 12 rats	10	Heart perforation Bleeding Cardiac insufficiency Anesthetics overdose Technique problems	1 1 2 4 2	2 (16.6%)	Late thoracic bleeding (lethal)
Series 2 18 rats	9	Bleeding Cardiac insufficiency Anesthetics overdose Perforation	1 6 1 1	9 (50%)	0
Series 3* 19 rats	4	Technic problems	4	15 (79%)*	0

NB In series 3, 19 rats were operated, but 4 died during or immediately after surgery on day 0 from technical causes (ventilation break), not linked with the surgery, and were excluded. So when the protocol is strictly applied, the operation success % may be considered as 100%

Table 1: Issues and complications of the proposed alternative method of heart lesion.

In series 3, out of the 19 involved animals, all the 15 ones strictly operated under the standard conditions defined from the previous work up, have survived and no early or late complication was observed. 12 of them were submitted to echocardiography at days 10+/-2 and 30+/-5 and immediately euthanized for histological examination at day 30 +/- 5.

ECG of a rat under anesthesia before and after heart lesion is presented (Figure 2A). Within 30-45 minutes after the lesion, the ECG has shown significant 30+/-5 millivolts mirror ST-modification in leads I and III, as well as slight enlargement (up to 20 millisecond) of QRS complex (Figure 2B). In late delays, typical for infarcted heart modifications of ECG were noted: deep Q wave (60-80 millivolts) and elevated ST (40-60 millivolts) in 2 leads (Figure 2C).

Control echocardiography provided on 11 different intact animals, as well as repeated on the same animal, has shown satisfying results considering either liability or reproducibility (Figure 3A). So they were used for comparison with those of operated animal investigations.

The results of the echocardiography provided on 12 animals of series 3 at days 10 +/- 2 and 30 +/- 5 and on the 11 intact controls (once each animal) have confirmed the functional significance of the lesion. Ejection fraction (EF) was significantly reduced (average EF decrease: 37.4%) in comparison with intact control ($p<0.001$), especially at the first delays 1-2 weeks (Figure 3B). Later some improvement but not complete normalization of the function seemed to occur (average EF decrease-27.3%) the difference with control remaining significant ($p<0.01$) (Table 3a,3b).

In late delays (2 months and more) the EF remained decreased (determined as about 50-60%). It is to be noted that the variability of the experimental results, especially at delay 10+/-2 days, was higher than the variability of control series data's.

The results of the heart wall thickness evaluation at the lesion level comparatively to the intact area and comparatively to the value obtained in the control animals are more difficult for interpretation because in early delays the wall limits determination is biased by tissue edema of the lesion zone. After 30 days, a non-significant tendency to reduction of the heart wall thickness in the lesion area was noted (Table 3c). In 2 cases it corresponded to aneurysm formation visually and histologically confirmed (Table 2 and Figure 4).

The histological evolution of the lesions was the following (Figure 4):

1) At day 0, within the first hours after operation, there was an important tissue edema, signs of myocytes suffering (shrunk nuclei, disappearance of muscle striation, intra-cellular edema, coagulation necrosis), activation of capillary endothelium and an inflammatory reaction start (Figure 4 a-4e).

2) During the following days 2, 4, 7, 10 both degenerative and inflammatory reactions increased. A coagulum indicated the place of cauterization.

3) At day 14: mild fibrosis was already present, and developed later at days 21 and 28 (Figure 4f).

Series 1

Date operation	Sex /strain	Age (month)	BW (g)	lesion dim. (mm)	Operation time (min)	Issue	Observ. time	Complications	Histo N°	investig
12.11.09	F/F	nm	203	2x1	65	†	D1	Thoracic bleeding	1241	
19.11.09	F/F	nm	243			†		Technic problems	0	
19.11.09	F/F	nm	206			†		Technic problems	0	
19.11/09	1M/F	~12	298			†	D0	Technicproblems	0	
03.12.09	M/W	nm	397	nm	30	†	D37	Thoracic bleeding	1248	
03.12.09	M/W	nm	403	nm	60	†	D0	Technic problems	0	
14.01.10	M/W	nm	360	2x2	10	Euth.	D42	Thoracic bleeding	1257	Echo D33
21.01.10	M/W	nm	340	deep	10-15	┼	D1	Perforation	1246	
09.02.10	M/F	nm	350	nm	40	†	D1	perforation	1256	
17.03.10	M/F	12	388	large	nm	†	D0	CRA	0	
05.08.10	M/F	nm	339	nm	15	†	D0	CRA	1277	
05.08.10	M/F	nm	344	nm	15	†	D0	CRA	0	
12	12									

Series 2

Date N°	Sex/ strain	Age (Mo)	BW (g)	lesion dim.	Oper. time	Issue	Observ. time	Complications	Histo	Investigation
14.05.13	M/W	12	438	3x5	38	┼	D0 3h	CRA	1379	EGG
07.05.13	M/W	nm	392	5	60	Euth.	7,5 mo	0		ECG
30.04.13	M/W	12	394	5	40	┼	D0 3h	CRA	1377	ECG
02.04.13	M/W	14	464	nm	20	┼	D0 35min	CRA	1368	ECG
26.03.13	M/W	13	452	1cm²	20	Euth.	6 mo	0	1400	ECG
22.01.13	M/W	10	434	8	30	┼	D0 3h20	Thoracic bleeding	1366	ECG
18.12.12	M/W	16	462	5	23	Euth.	D21	0	1365	ECG
11.12.12	M/W	18	421	nm	27	Euth.	D7	0	1364	ECG
04,12,12	M/W	nm >12	434	5	20	Euth.	D14	0	1363	
06.11.12	M/W	nm>12	387	>5	20	┼	D0 1 h.	anesth	0	
16.10.12	F/W	nm	234	nm	13	┼	D0 1h30	CRA	0	
09.10.12	F/W	nm	245	nm	20	Euth.	D35	0	1358	
13.09.12	M/W	nm	440	5x5	30	┼	D01h40	CRA	1355	
28.08.12	M/F	16	403	nm	25	Euth.	D35	0	0	
31.07.12	M/F	nm	420	3x5 deep	~ 20	Euth.	D2	0	1352	
05.11.13	F/W	8	250	Trans mural	17	┼	D0	Perforation		ECG
12.11.13	F/W	8	250	10x10x2	25	┼	D0 4h	CRA	1402	Echo
03.12.13	M/W	5	364	8x8x2	35	Euth.	8 mo	0	1487	Echo D 52, 8 mo
18									18	

Series 3											
Date N°	**Sex /strain**	**Age (mo)**	**BW (g)**	**lesion dim. (mm)**	**Oper. time (min)**	**Issue**	**Observ. time**	**Complication**	**Histo**	**Inves tiga tions**	**Remarks**
0312 14	M/W	4	364	9x9	35	Euth.	7 mo	0	1487	Echo 2 & 5 mo	
18.02..14	M/W	nm	452	8x7	28	death	D0 50 min	CRA	1453		Ventil. .tech. probl
18.12. 14	M/W	8	461	8x7	death	J0 3 h	D 0 3 h	CRA	1454	ECG	Tech. probl.
20.02. 14	M/W	7,5	395	nm	30	Euth.	5 mo D141	0	1488	Echo 5 mo	
25.02. 14	M/W	nm	420	Nm	20	death	D0 2h	CRA	1455	ECG	Import. infarct
28.02..14	M/W		480	<1 cm²		Euth.	D30	0		Echo D4&30	
04.03. 14	M/W	10	445	Nm	20	death	D0 1h	CRA	1456	ECG	Ventil. tech. probl.
11.03. 14	M/W	11	420	9x8	25	Euth.	D30	0	1466	Echo 10.30	
18..03 .14	M/W	11	441	9x9	15	Euth.	D30	0	1468	Echo 10,30	
25..03. 14	M/W	11	394	<1 cm²	27	Euth.	D30	0	1469	Echo 10,30	
01.04. 14	M/W	11.5	421	7x8	25	Euth.	D41	0	1471	Echo 10,30	Aneu rysm
08.04. 14	M/W	10	394	5x5	28	Euth.	D34	0	1470	Echo 10,34	
15.04. 14	M/W	10	457	5.5x4	29	Euth.	D29	0	1472	Echo 8,29	
06.05. 14	M/W	11	433	8x7	30	Euth.	D24	0	1473	Echo 8,24	
20.05. 14	M/W	10	433	8x8	20	Euth.	D31	0	1478	Echo 10,31	Aneu rysm
27.05 14	M/W	10	426	2x9	23	Euth.	D30	0	1484	Echo 0,7,30	
26.01. 15	M/W	8	404	8x8	20	living					
23.06. 15	M/W	7	434	7x8	15	living					
24.07. 15	M/W	7	440	8x8	35	living					
Total 19											

NB: CRA – cardio-respiratory arrest; echo. – echography; Euth. - euthanasy; Histo – histology, tech probl – technique problems

Table 3: Results of ultrasounds investigations (echocardiography) (EF: ejection fraction; RC: heart rate; LVWT: left ventricle wall thickness, s in systole, d in diastole. M+/-SD on n=12).

4) After 1 month, the coagulum was practically phagocytized (Figure 4g); the fibrosis zone was well organized with oriented fibers, sometimes penetrating the healthy part of the myocardium (Figure 4 h-4j). In several cases the fibrosis was transmural and an aneurysm was formed. In 2 cases formation of cartilage into the trabecular muscle was observed in the neighboring of the lesion (Figure 4k-4l). At a small distance from the injured zone and in the remained intact parts of the heart, the myocardium looked quite normal. Later up to 8 months marked fibrosis remained at the lesion area.

Other organs – lungs and liver – have shown no sign of evident pathology.

Discussion and Conclusion

These data suggest that an experimental technique and model of precise, reproducible, visually controlled lesion of the myocardium wall has been developed and specified. Precision was warranted by visual control of the technique, reproducibility was proved by standardization of the procedure. This has led to stable results of surgery itself, of functional follow up investigations data and of histological findings. It is to be noted that the success rate was low at the beginning of the research (16%) and much better during the last period of time (up to 100%), when the optimal experimental conditions were defined. Indeed, as well as coronary artery ligation, the procedure remained delicate, but was rather well tolerated, if the surgical technique and anesthesia conditions* were respected. It does not seem technically more difficult than interventions on the coronary arteries themselves, and it needed only usual equipment for cardiac surgery.

Another advantage of the proposed technique is the possibility of follow up with reliable functional evaluation of the damage by ultrasound investigation. In our experiments some difficulties of measurements were due to the high rate beatings heart of the rat that may be overcome by the use of a better resolution ultrasonic probe. Peculiarities of EF determination by Simpson method has to be lined: depending on localization of the ventricle section chosen for surface and volume calculation - through intact or injured part of the heart-, the results may differ. That was taken into account during the results analysis. Nevertheless echocardiography data have always shown a high correlation with anatomy and histology ones.

An interesting histological finding has also to be lined: the presence of cartilage in muscular cords of the left ventricle in some cases 1 or more months after lesion.

Such observations were previously mentioned after stem cells implantations and explained by multi potentiality of stem cell development including cartilage or even bone formation. Here no implantation of any tissue was performed. Thus, we can only speculate that cardiomyocytes could de-differentiate and give rise to chondroblast

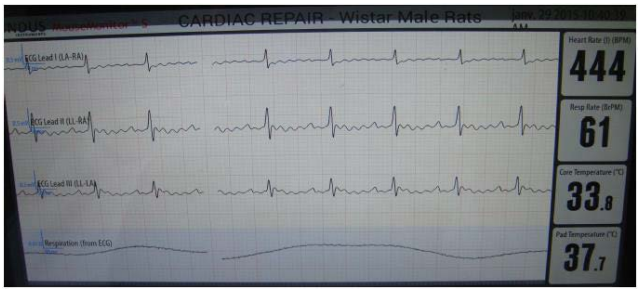

Figure 2A: Electrocardiography of the rat : Before operation (control).

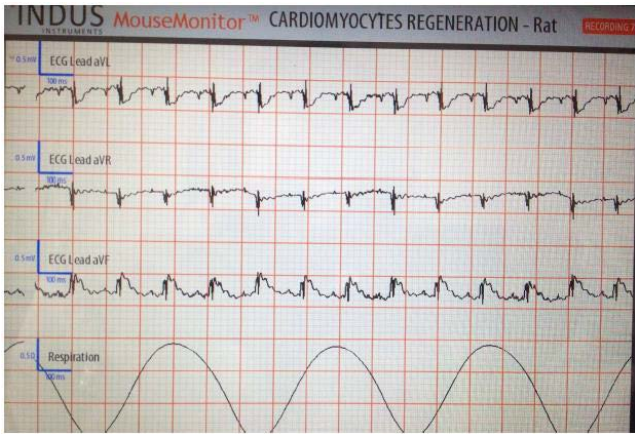

Figure 2B: Electrocardiography of the rat: 30 minutes after the myocardial lesion: mirror significant changes (more than 2 divisions, i.e. 30 millivolts) of ST in leads I and III, slight enlargement of QRS complex (up to 25-30 milliseconds).

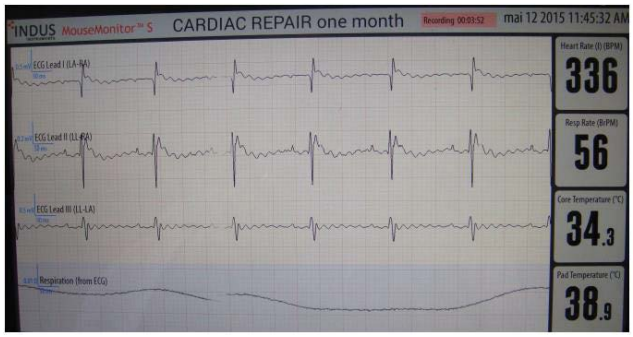

Figure 2C: Electrocardiography of the rat : within 1 month after operation: deep Q wave in lead 1, elevated ST in lead III. The animals are under anesthesia and in the same conditions of room temperature.On the screen from above to lower part: leads I, II, III, breath registration; on the right: heart rhythm, breath rhythm, rectal temperature and given temperature of table on which the rat is laying. Value of a small division on the screen: 10 or 20 millisecond (abscissa) and 10 or 20 millivolt (ordinate).

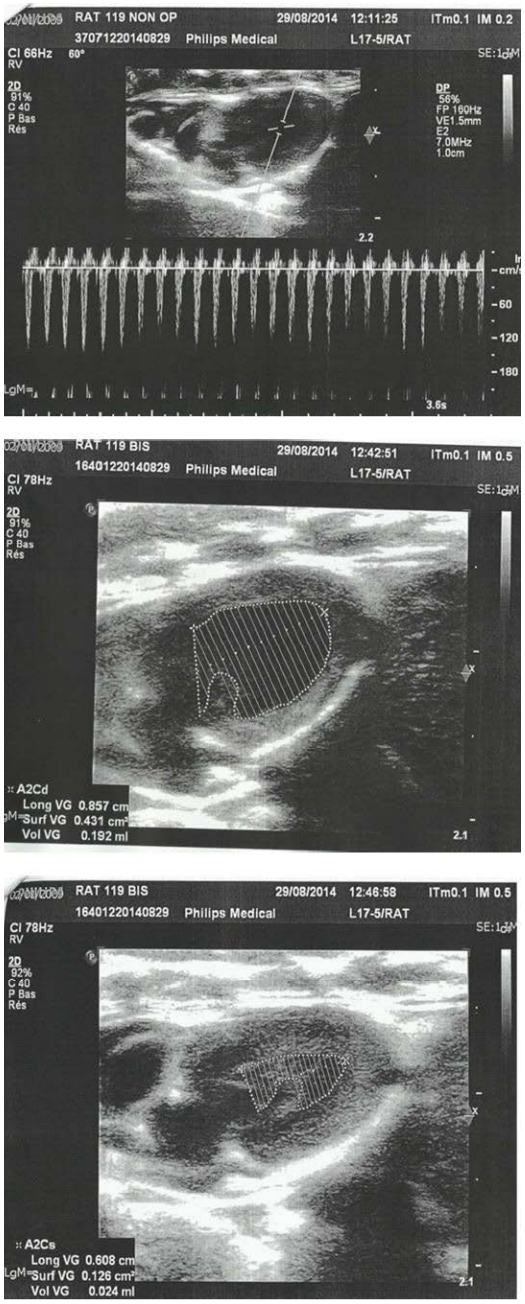

Figure 3A: Ultrasound investigation after high temperature myocardial lesion.Control intact animal: Cardiac rhythm and left ventricle longitudinal section surface in systole and diastole (EF 80%).

precursors, or that local and circulating stem cells were involved, perhaps under the influence of inflammatory cytokines, local hypoxia due to infarction or toxins and of the contractile function of the heart muscle, as assessed in literature [24-28]. Another explanation of the observed cartilage formation could be that fibroblast metaplasia could develop under the influence of mechanic and hypoxia conditions.

Anyway, the technique seems also to be a robust model of chronic cardiac insufficiency thanks to a long lasting EF decrease explained by local myocardium fibrosis up to possibility of aneurysm formation.

Besides, whole heart histology has detected significant variations in thickness of the ventricle walls - kinds of crypts and trabeculae – between the bases of muscular part of the atrio-ventricular valves. Ultrasound investigation has also pointed a significant difference between systole and diastole ventricular thickness (Table 3). This justifies the necessity:

a) of controlling the lesion maximal deepness limit as no more than 1 mm,

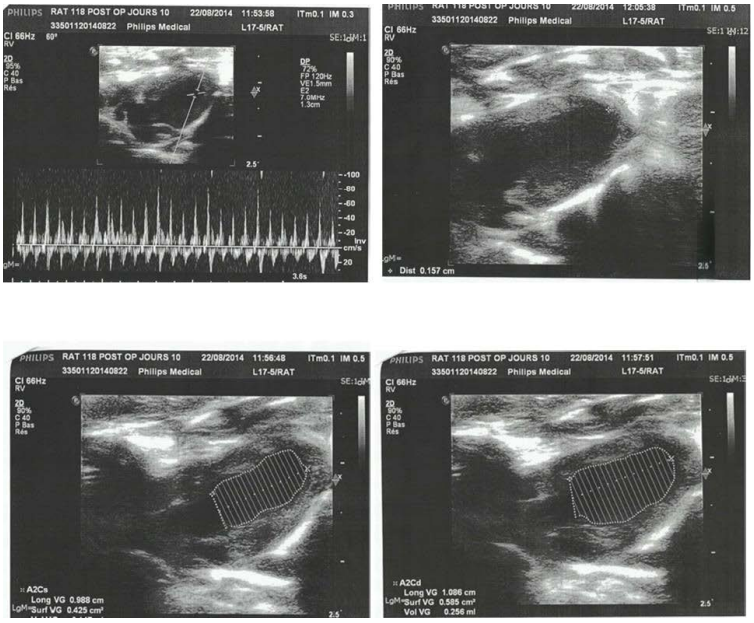

Figure 3B: Ultrasound investigation after high temperature myocardial lesion.Operated animal at day 10: left ventricle longitudinal section surface significant decrease of the difference between systole and diastole (EF 50%).

b) of ensuring a visual control of the safeness of the myocardium inner layer during tip applications for avoiding perforation.

The last advantage of our technique is the rather strong limitation of injured area, confirmed by ultrasound and histological evaluations: the extension of the injured – shocked – zone was no more than 1 mm around the necrotic area (Figure 4b-4f); and the presence close to the lesion of healthy or only slightly affected myocardium, the extension of inflammatory reaction and, later, fibrosis being limited (Figure 4d-4j). This may enhance the development of eventually grafted cells close to the lesion.

The negative aspects of the purposed technique may be the presence of a coagulum of burnt tissues in the lesion zone not only immediately after the operation but also within several weeks later, in fact as remnants only. The inflammatory reaction directed to the destruction and phagocytosis of this "foreign material" may be a danger for implanted in the neighboring area tissues, or an enhancing factor, as mentioned above, since vascularization of the zone may be increased. This must be verified in further experiments with stem or precursor cells implantation. Besides, knowledge of the different steps of the lesion healing allows study the most favorable moment for intervention by cell therapy.

In the other described models of cardiac lesion, for instance after coronary artery occlusion, the determination of the resulting injury extent needs special sophisticated techniques [21,22]. The same inconvenient of large involvement of the heart structures and even of the whole organism is obvious when lesions are caused by injection of drugs [17,18,23]. Ferric chloride use is not compatible with MRI imaging investigation, necessary to determine the lesion extent and evolution. Radiofrequency has been used recently to induce cardiac lesions through induction of intravascular coagulation, with promising results [16]. It represents a non-invasive coronary arterial occlusion with the same limits as mentioned above.

In conclusion, our technique is reproducible and, with experience, allows animals survival for a period, which is long enough to investigate the natural evolution of cardiac function after a localized lesion. It will be used to investigate the best modalities for fetal heart or cultured cardiomyoblast implantation to improve the injured heart remodeling and enhance tissue regeneration.

The early activation of regenerative processes after the thermic lesion of the heart may suggest that early cardiomyoblast implantation close to the lesion could have some chances of success.

Testing fetal heart implantation using our technique of local heart injury might be susceptible to answer to the above-mentioned question (concerning the cause of cell therapy controversial results) and give precious indications for reparative heart surgery. The first results obtained in our further investigation seem to support this hypothesis [24-28].

*Some other conditions of success are: an adapted ventilation control, avoiding diaphragm and phrenic nerve lesion, accurate hemostasis, rectal temperature control (no less than 32.5°C) during anesthesia, operation duration no more than 30-40 min.

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Control animal	Investigation /measure number	RC (M+/-SD)	EF (M+/-SD) N=5	EF (M+/-SD) N=12	EF variability N=5 // N=16
Rat n°1	5 / 12	406 +/- 31	69.6 +/- 2.2	69.4 +/- 5.0	3.1% // 7.2%
Rat n°2	5 / 16	382 +/- 26.6	69.5 +/- 3.3	70.4 +/- 6.0	4.7% // 8.6%

No statistic difference between results (p>0.05) and acceptable variability (< 10%)

3a: Evolution of repeated EF evaluation in 2 control animals (for liability assessment)

Delay (days)	N° of animals	RC (M+/-SD)	EF (M+/-SD)	% EF average decrease	Results variability %
10 ± 2	**11**	421 ± 35	45.6 ± 13.4 **	37.4	17.4
30 ± 5	**11**	385.5 ± 50	56.3 ± 10.2 *	27.3	15.4
Control	**11**	395 ± 30	72.9 ± 5.3		7.3

*p<0.01 comparatively to control

**p<0.001 comparatively to control

Acceptable variability, better in control than after lesion, especially in early delay

3b: Evolution of EF in operated and intact (control) animals (the same 11 operated animals being investigated at 2 delays).

Series	systole normal wall	systole injured part	diastole normal wall	diastole injured part	Animal number
At day 10	3.28+/-0.26	nm	2.13+/-0.43	nm	9
At day 30	3.47+/-0.33	3.07+/-0.38	2,66+/-0.26	2.2 +/- 0.42	9
control	3.47+/-0.27	-	2.46+/-0.31	-	11

No statistic difference between injured part and normal wall at any moment (p>0.05).

3c: Results of left ventricle wall thickness (LVWT) measurements (M+/-SD).

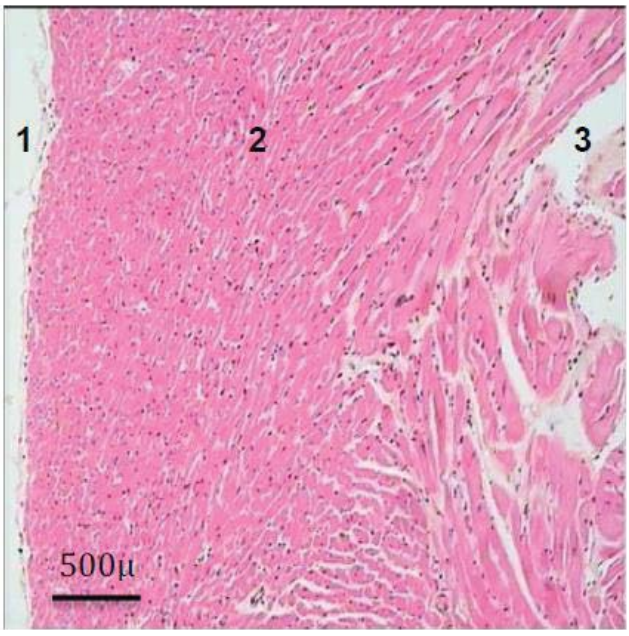

Figure 4A: Histological aspects of heart high temperature lesion:Normal heart: Epicardium (1), myocardium (2), endocardium (3) covering the trabecular/muscular columns of the valve fibrous strings or chordae tendineae (hematoxilin eosin x5).

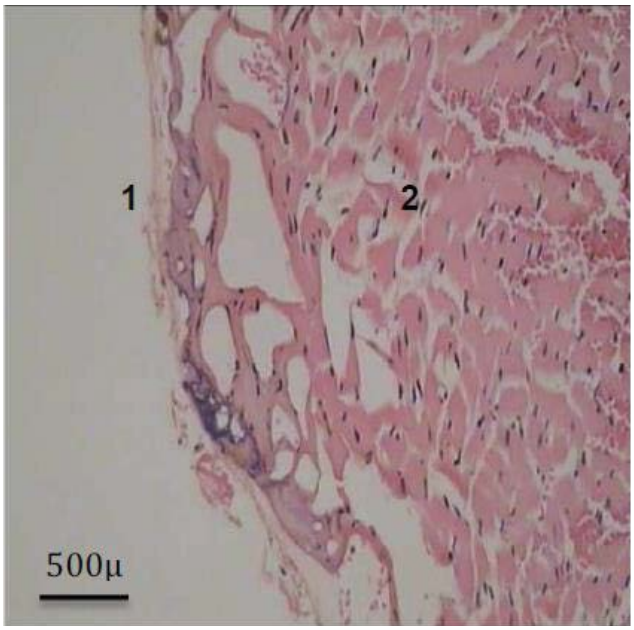

Figure 4B: Histological aspects of heart high temperature lesion.Day 0 after lesion: In the lesion center, epicardium and sub epicardial lesion in form of bubbles due to cauterization heat (hematoxilin eosin x5).

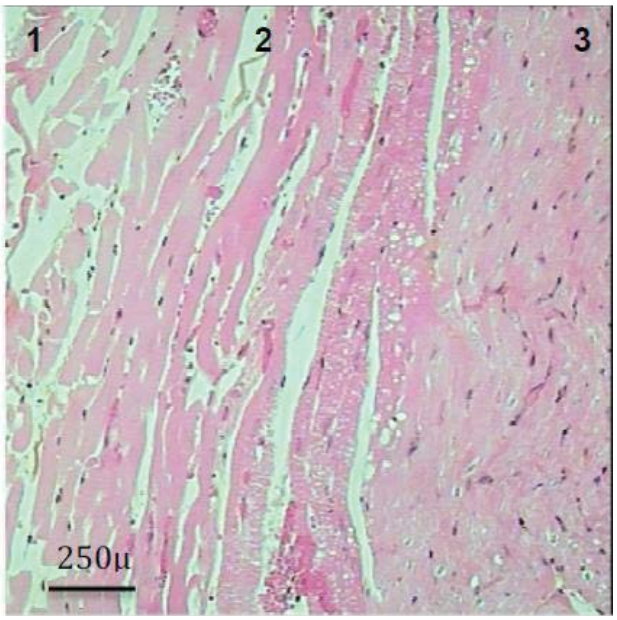

Figure 4C: Histological aspects of heart high temperature lesion.Day 0 (4 hours): Superficial area (1) shows a coagulation necrosis with loss of cardiomyocyte nuclei, intermediary area (2) shows a coagulation necrosis with cardiomyocytes vacuolization and inner area (3) with an apparently preserved myocardium (hematoxilin eosin x 10).

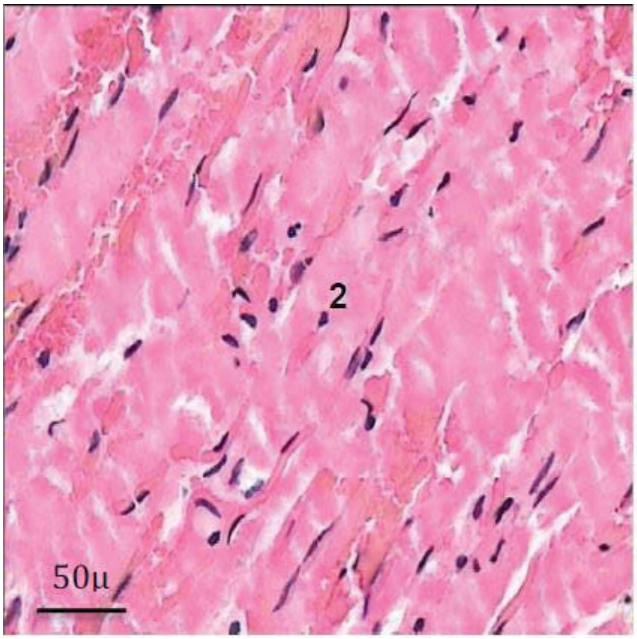

Figure 4D: Histological aspects of heart high temperature lesion.Day 0 (4hours): Cardiomyocytes nuclei alterations focused (hematoxilin eosin x40).

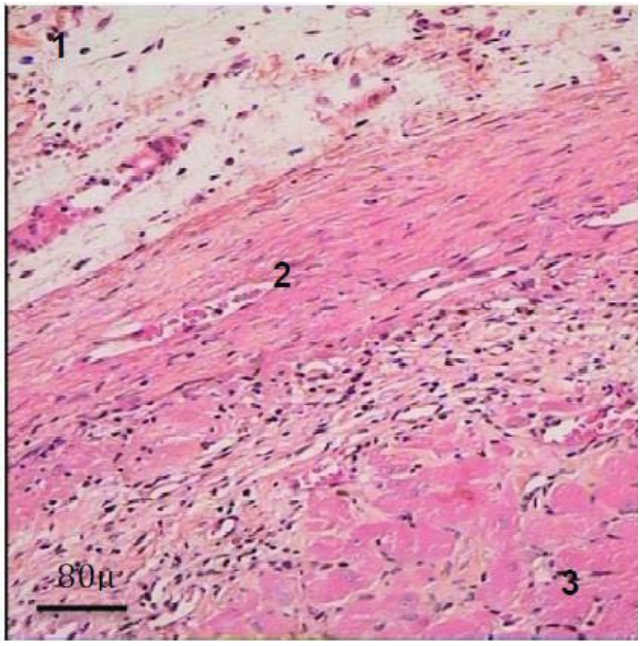

Figure 4F: Histological aspects of heart high temperature lesion.Day 14: Necrosis in superficial area (1), mild fibrosis: Inflammatory reaction and beginning fibrosis in the intermediary area (2) and preserved cardiomyocytes in deep area (hematoxilin eosin x20).

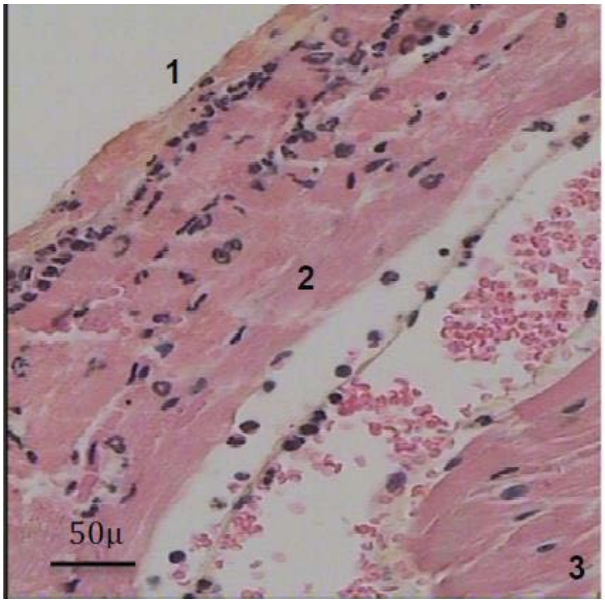

Figure 4E: Histological aspects of heart high temperature lesion.Day 0 (6 hours): Coagulation necrosis with neutrophil infiltration (hematoxilin eosin x20).

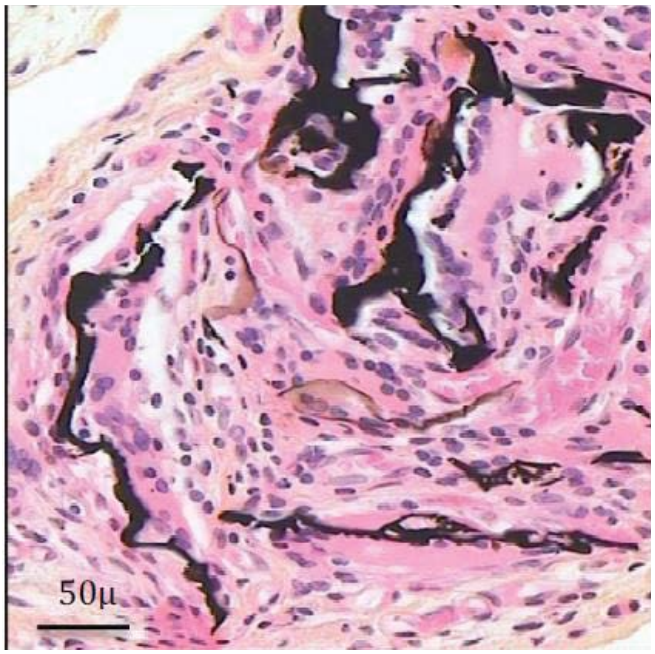

Figure 4G Histological aspects of heart high temperature lesion.Day 29: Resorption inflammatory reaction of coagulum remnants with giant cells in a small burnt sub epicardium area (hematoxilin eosin x20).

References

1. World Health Organization (2004) Department of Health Studies and Informatics in the Information. Evidence and Research Cluster. The Global Burden of Disease. 2004 Update. Geneva, Switzerland.
2. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, et al. (2012) Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 125: 188-197.
3. Cowye HR (1999) An annotated references in epidemiology. Europ J Heart Failure 1: 101-102.
4. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, et al. (2002) ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary. J Heart Lung Transplant 21: 189-203.

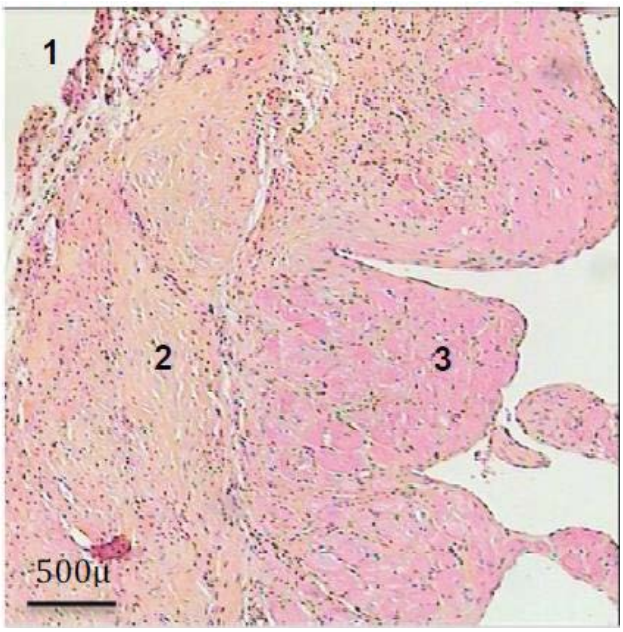

Figure 4H Histological aspects of heart high temperature lesion. Day 30: General view of the lesion area: heart apex (1), muscular columns of the valve tendineous strings (hematoxilin eosin x5).

1. Epicardium with inflammatory cells,
2. Large cicatricial zone (50 % of the thickness of the external wall),
3. Partially preserved muscular columns.

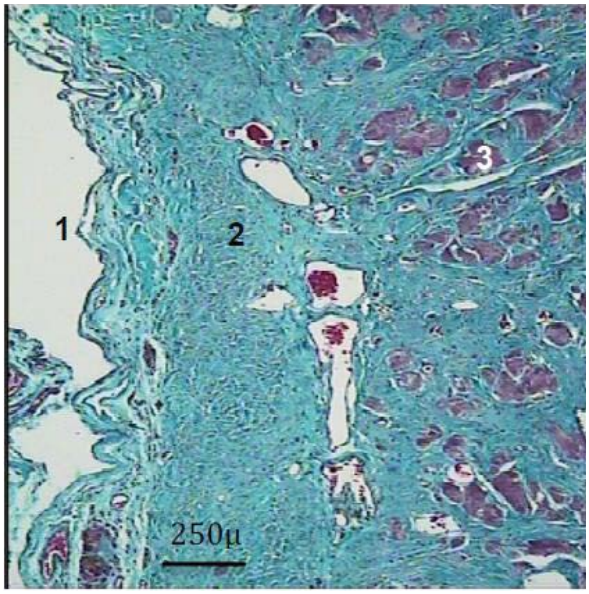

Figure 4I: Histological aspects of heart high temperature lesion: Day 34: The special coloration shows spider-like extension of the fibrosis. 1: epicardium, 2: fibrosis area, 3: intermediary area (Masson's Green Trichroma, x 5).

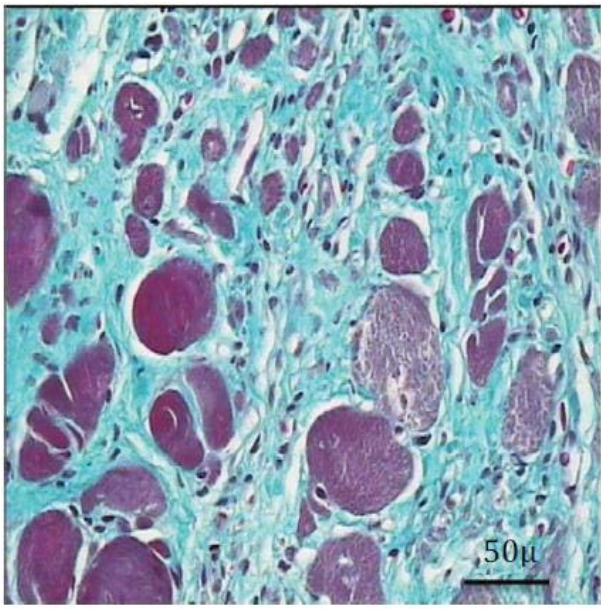

Figure 4J: Histological aspects of heart high temperature lesion.Day 30: Dissociation of the cardiomyocytes by fibrosis (Masson's Green Trichroma x 40).

Figure 4K: Histological aspects of heart high temperature lesion.Day 30: General view of one case of complete transparietal fibrosis (<->) of the apex with pillar involvement and aneurysm forming (hematoxilin eosin x5).

5. Rosso T, Malvezzi M, Bertuccio P, Negri E, La Vecchia C, et al. (2012) Cancer mortality in Italy, 2008, and predictions for 2012. Tumori 98: 559-567.
6. Delahaye F, Roth O, Aupetit JF, de Gevigney G (2001) [Epidemiology and prognosis of cardiac insufficiency]. Arch Mal Coeur Vaiss 94: 1393-1403.
7. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, et al. (2003) Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc Natl Acad Sci U S A 100: 7808-7811.
8. Etzion S, Battler A, Barbash IM, Cagnano E, Zarin P, et al. (2001) Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in a rat model of extensive myocardial infarction. J Mol Cell Cardiol 33: 1321-1330.
9. Yao M, Dieterle T, Hale SL, Dow JS, Kedes LH, et al. (2003) Long-term outcome of fetal cell transplantation on postinfarction ventricular remodeling and function. J Mol Cell Cardiol 35: 661-670.
10. Sakakibara Y, Tambara K, Lu F, Nishina T, Nagaya N, et al. (2002)

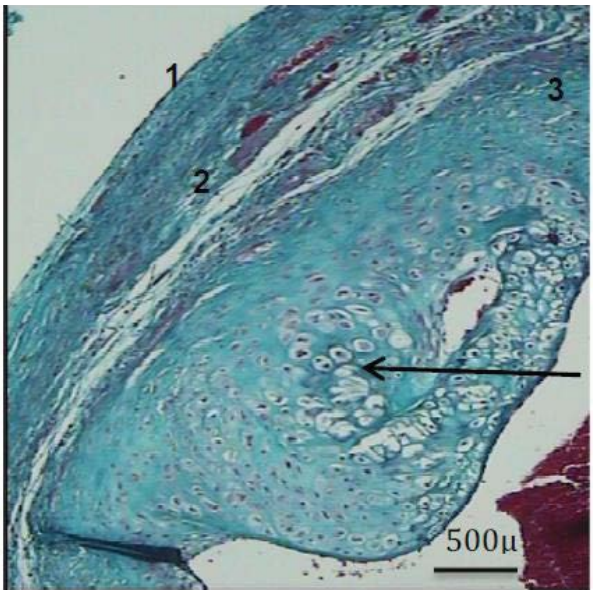

Figure 4L: Histological aspects of heart high temperature lesion:Day 30: Fibrosclerosis of the apex myocardium with chondrocyte metaplasia (Masson's Green Trichroma x 5).

Cardiomyocyte transplantation does not reverse cardiac remodeling in rats with chronic myocardial infarction. Ann Thorac Surg 74: 25-30.

11. Li RK, Jia ZQ, Weisel RD, Mickle DA, Zhang J, et al. (1996) Cardiomyocyte transplantation improves heart function. Ann Thorac Surg 62: 654-660.
12. Tucker DC, Snider C, Woods WT Jr (1988) Pacemaker development in embryonic rat heart cultured in oculo. Pediatr Res 23: 637-642.
13. Coulic V, DeKoster E, Delrée P, Deltenre P, DePrez C, et al. (2005) Experimental comparative evaluation of the functional capacities of ectopically grown foetal organs. Russ J Physl 91: 408-430.
14. Jaquet K, Krause KT, Denschel J, Faessler P, Nauerz M, et al. (2005) Reduction of myocardial scar size after implantation of mesenchymal stem cells in rats: what is the mechanism? Stem Cells Dev 14: 299-309.
15. Rodriguez LM, Leunissen J, Hoekstra A, Korteling BJ, Smeets JL, et al. (1998) Transvenous cold mapping and cryoablation of the AV node in dogs: observations of chronic lesions and comparison to those obtained using radiofrequency ablation. J Cardiovasc Electrophysiol 9: 1055-1061.
16. Crastina A, Pokreisz P, Schnitzer JE (2014) Experimental model of transthoracic, vascular-targeted photodynamically induced myocardial infarction. Amer J Physiol 306: 1182-1191.
17. El-Neweshy MS (2013) Experimental doxycycline overdose in rats causes cardiomyopathy. Int J Exp Pathol 94: 109-114.
18. Tanaka T, Sato R, Kurimoto T (2000) Z-335, a new thromboxane A(2) receptor antagonist, prevents arterial thrombosis induced by ferric chloride in rats. Eur J Pharmacol 401: 413-418.
19. Vivaldi MT, Kloner RA, Schoen FJ (1985) Triphenyltetrazolium staining of irreversible ischemic injury following coronary artery occlusion in rats. Am J Pathol 121: 522-530.
20. Masini E, Gambassi F, Giannella E, Palmerani B, Pistelli A, et al. (1989) Ischemia-reperfusion injury and histamine release in isolated guinea-pig heart: the role of free radicals. Agents Actions 27: 154-157.
21. Greve G, Saetersdal T (1991) Problems related to infarct size measurements in the rat heart. Acta Anat (Basel) 142: 366-373.
22. Ohtani H, Callahan RJ, Khaw BA, Fishman AJ, Wilkinson RA, et al. (1992) Comparison of technetium-99m-glucarate and thallium-201 for the identification of acute myocardial infarction in rats. J Nucl Med 33: 1988-1993.
23. Nakaoka H, Nakagawa-Toyama Y, Nishida M, Okada T, Kawase R, et al.(2013) Establishment of a novel murine model of ischemic cardiomyopathy with multiple diffuse coronary lesions.
24. Iehoczky-Mona J, Mccandless EI (1964) Ischemic induction of chondrogenesis in myocardium. Arch Pathol 78: 37-42.
25. Fishbein MC, Maclean D, Marokko PR (1978) Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. Am J Pathol 90: 57-70.
26. Hollander CF (1968) Cartilaginous focus at the base of the non-coronary semilunar valve of the aorta in rats of different ages. Exp Gerontol 3: 303-307.
27. Boor PJ, Ferrans VJ (1985) Ultrastructural alterations in allylamine cardiovascular toxicity. Late myocardial and vascular lesions. Am J Pathol 121: 39-54.
28. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, et al. (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 107: 1395-1402.