

## Research Article

# Comparison of Myocardial Remodeling between Cryoinfarction and Reperfused Infarction in Mice

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Myocardial infarction is associated with inflammatory reaction leading to tissue remodeling. We compared tissue remodeling between cryoinfarction (cMI) and reperfused myocardial infarction (MI) in order to better understand the local environment where we apply cell therapies. Models of closed-chest one-hour ischemia/reperfusion MI and cMI were used in C57/Bl6-mice. The reperfused MI showed rapid development of granulation tissue and compacted scar formation after 7 days. In contrast, cMI hearts showed persistent cardiomyocyte debris and cellular infiltration after 7 days and partially compacted scar formation accompanied by persistent macrophages and myofibroblasts after 14 days. The mRNA of proinflammatory mediators was transiently induced in MI and persistently upregulated in cMI. Tenascin C and osteopontin-1 showed delayed induction in cMI. In conclusion, the cryoinfarction was associated with prolonged inflammation and active myocardial remodeling when compared to the reperfused MI. These substantial differences in remodeling may influence cellular engraftment and should be considered in cell therapy studies.

## 1. Introduction

The experimental models of myocardial infarction have largely contributed to a better understanding of the pathophysiology of myocardial infarction. Studies on large animal models revealed many mechanisms involved in myocardial injury and repair [1, 2]. In the last few years, transgenic mice and gene targeting technologies allowed profound studies of the underlying pathomechanisms. For this purpose, we developed murine models of myocardial ischemia and reperfusion and used them to study the mechanisms involved in ischemic myocardial injury [3, 4]. We described pathological features of reperfused myocardial infarction in mice, that is, rapid formation of granulation tissue and subsequent development of a stable scar [4]. Our studies also revealed an important role for macrophages and their associated inflammatory and remodeling-related

mediators [5, 6]. A profound understanding of these mechanisms is crucial for projects utilizing novel cellular therapies, since the local microenvironment seems to exert a strong influence on the cells applied in our model of myocardial cryoinfarction [7–9].

This study compares the pathology and the sequence of cellular and molecular events in the two mechanistically different lesion models of myocardial cryoinfarction and reperfused infarction and reveals substantial differences in myocardial remodeling between them. Both models show a transient inflammatory response associated with induction of chemokines, cytokines, and remodeling-related mediators. The cryolesioned heart showed a prolonged remodeling with postponed development of granulation tissue and scar formation, which was associated with persistent macrophage infiltration in the injured tissue when compared to reperfused infarction.

## 2. Material and Methods

**2.1. Study Animals.** Wild-type C57/Bl6-mice (Charles River, Sulzfeld, Germany) of 18 to 25 g weight and 8 to 10 weeks old were used in our infarction models. Group size in reperfused infarction model was  $n = 8$  mice and in cryoinfarction  $n = 6$  mice. All experiments were performed in accordance with an animal protocol approved by the local governmental authorities.

**2.2. Mouse Model of Reperfused Myocardial Infarction.** In an initial surgery, mice were anesthetized with pentobarbital i.p. (2.5  $\mu\text{g/g}$ ; Merial, Halbermoos, Germany) as previously described [10]. The thorax was shaved and disinfected with betadine. Then, mice were intubated with a PE-90 tube (Becton Dickinson, Sparks, MD, USA) connected to a small animal respirator (Rodent Ventilator Mod. 681, Harvard Apparatus Inc., Millis, MA) and ventilated at a frequency of 110/min with tidal volume of 0.25 mL. Animals were kept anesthetized using isoflurane concentration between 0.8 and 1.3 vol-%. The chest was opened by left parasternal thoracotomy. Pericardium was dissected and partially removed, and a Prolene 8-0 suture (Ethicon, Norderstedt, Germany) was placed around the left descending coronary artery (LAD). Suture ends were threaded through a sterile PE-10 tube (Becton Dickinson) of 3 mm of length, exteriorized through the thoracic wall, and then stored subcutaneously. The thorax was closed with interrupted Prolene 6-0 stiches, and the skin was closed with a running Prolene 6-0 suture. At the end, metamizol (100 mg/kg; Novalgin) was given for analgesia in a mixture with cefuroxim as antibiotic prophylaxis i.p. (100 mg/kg, Zinacef; Bristol-Myers Squibb, Munich, Germany). Mice were allowed to recover for 7 days from trauma of initial surgery. Then, myocardial infarction was induced under isoflurane anesthesia as described before [4]. The skin was reopened, and the ends of the LAD-ligature were connected to heavy metal picks. Pulling on the ligature ends induced LAD-occlusion, and this ischemia was maintained for 60 minutes. After removal of the LAD-ligature, the blood flow was restored in the reperfusion. ECG monitoring of Einthoven lead II during and after LAD-occlusion confirmed successful ischemia and reperfusion. Mice with nonpersistent ST-elevation were excluded from further evaluation. Postoperative analgesia and antibiotics were administered i.p. as mentioned above. One-hour ischemia was followed by reperfusion for 6 hrs, 1, 3, 7, and 14 days. Mice were euthanized using an overdose of pentobarbital i.p.; hearts were excised and fixated in zinc-paraformaldehyde (Z-fix, 4%; Anatech, Battle Creek, MI, USA) for histology or stored in RNA-later solution (Qiagen, Hilden, Germany) for mRNA-studies.

**2.3. Mouse Model of Cryoinfarction.** Animals were sedated by brief exposure to narcotic gas containing 50% O<sub>2</sub>, 50% N<sub>2</sub>O, and 3 to 4 vol-% of isoflurane as previously described [11]. Thereafter, mice were positioned on a temperature-controlled plate (37°C) in supine position, intubated, and

ventilated using a small animal respirator (Harvard Apparatus) as described in the previous section. Anesthesia was maintained with isoflurane concentration between 0.8 and 1.3 vol-%. Then, the left thoracic wall was shaved and disinfected with 70% isopropanol. An anterolateral skin incision was performed 5 mm above the costal margin, the anterolateral thoracic muscles were transected, and the thorax was opened in the fifth intercostal space. The pericardium was opened, and the apex of the heart was exposed. Cryocoagulation was performed by placement of a copper probe (3 mm diameter, cooled in liquid nitrogen for 2 min) to the free left ventricular wall (3 times for 20 seconds) in order to achieve reproducible, large transmural myocardial lesion. After induction of the cryoinfarction, a 22-G chest drain (Optiva, Johnson & Johnson, New Brunswick, NJ, USA) was inserted into the left pleural cavity to prevent pneumothorax. The thorax was closed with Prolene 6-0 sutures, and air was drained using negative pressure on chest drain before extubation. Metamizol and cefuroxime were given i.p. after the surgery. At the end of the protocol, the hearts were excised after the above-mentioned time periods and further processed for histological and molecular analysis.

**2.4. Histology.** After excision, hearts were washed in cardioplegic solution containing 4 g NaCl, 3.73 g KCl, 1 g NaHCO<sub>3</sub>, 2 g glucose (all from Berlin Chemie, Berlin, Germany), 3 g 2,3-butandion monoxime (Sigma-Aldrich, Munich, Germany), 3.8 g ethylenglycol tetra acetic acid (Sigma), 0.2 mg nifedipine (Sigma), and 10 ml heparin (1000 IU/ml; Ratiopharm, Ulm, Germany), all of which were dissolved in 1 L of isotonic NaCl (Berlin Chemie). Blood remnants were washed out of the ventricles. Hearts were fixated in zinc-paraformaldehyde for 24 hrs and further processed using standard paraffin embedding. Hearts were cut from basis to apex, and at every 250  $\mu\text{m}$ , a set of ten 5  $\mu\text{m}$  sections were mounted on glass slides [3]. The first section was stained with hematoxylin and eosin for initial evaluation. Myocardial sections below insertion of the papillary muscles were further used for histology and immunohistochemistry. Photographic images were recorded on a computer system equipped with a digital camera (DP70, Olympus, Hamburg, Germany), and planimetric evaluation was performed using ANALYSIS software (Olympus). Picosirius red staining of total collagen was used to evaluate development of fibrosis in the myocardial scar. Cell density was measured by cell count of immunohistochemically stained cells in infarcted myocardium and was expressed as number per mm<sup>2</sup>. We used the following primary antibodies for immunohistochemistry: alpha smooth muscle actin mouse monoclonal antibody (clone 1A4; Sigma, St. Louis, MO, USA) for myofibroblasts, F4/80 rat monocyte/macrophage antibody (Serotec, Duesseldorf, Germany) for macrophages and MCA 771 G rat monoclonal antibody for neutrophils (Serotec). Furthermore, samples were stained for macrophage maturation marker osteopontin-1 using a goat polyclonal antibody (P-18, Santa Cruz, Heidelberg, Germany) and for macrophage elastase using a rabbit monoclonal anti-MMP12 antibody (Abcam, Cambridge, UK). Immunohistochemical

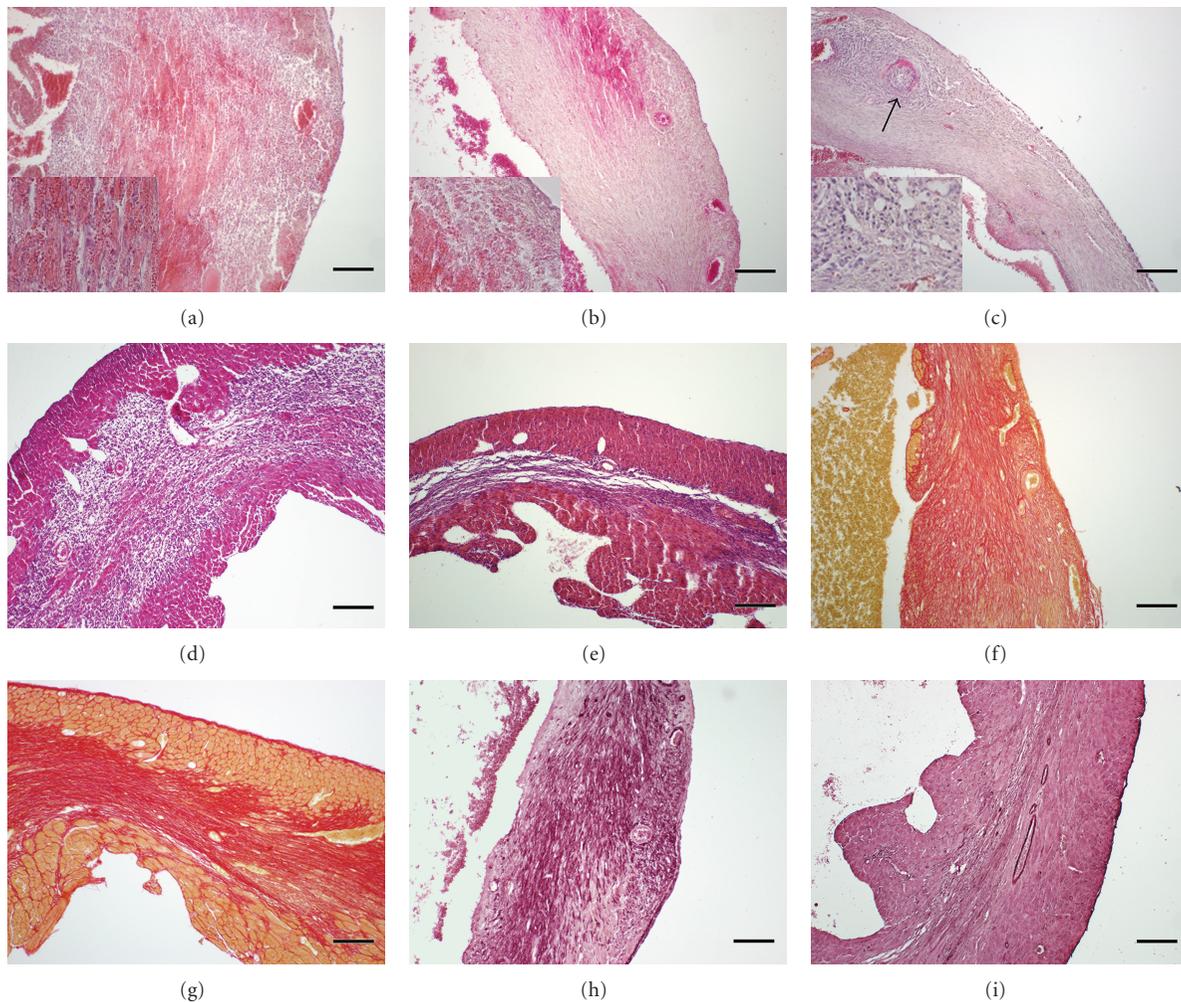


FIGURE 1: Comparison of myocardial histopathology between cryoinfarction and reperfused infarction. Hematoxylin-eosin staining of left ventricular lateral wall in cryoinfarction after (a) 3 days an increased cellular infiltration with extensive cardiomyocyte debris, with (b) formation of granulation tissue after 7 days, and (c) an almost compacted scar formation after 14 days postinjury with permanent occlusion of a large coronary vessel (arrow). In parts (a)-(c), a higher magnification (400x) insert shows cellular and matrix content in detail. In contrast, (d) reperfused myocardial infarction shows granulation tissue formation after 3 days and (e) a compacted, nontransmural scar formation after 7 days reperfusion. Picosirius red staining of (f) cryoinfarction after 7 days reveals loose collagen fibers, while (g) reperfused infarction presents a compacted, collagen-rich scar. Alpha smooth muscle actin staining of myofibroblasts (black-stained cells) indicates (h) active interstitial remodeling 7 days after cryoinfarction whereas (i) reperfused infarction has only few positive cells in the scar (eosin counterstaining). Scale bar: 150  $\mu$ m.

staining was performed using appropriate Vectastain Elite ABC kits and diaminobenzidine (AXXORA, Loerrach, Germany). M.O.M immunodetection kit (AXXORA) was used for mouse-derived antibodies.

**2.5. RNA Extraction and Taqman RT-qPCR.** Excised hearts were cleared of large vessels and atria and placed in RNA-later solution (Applied Biosystems, Foster City, CA, USA). Hearts were minced using a tissue tearor (Tissue Tearor Modell 398; Biospec, Bartlesville, OK, USA), and mRNA was isolated using standard phenol/chloroform extraction method (Trizol, Applied Biosystems). mRNA was transcribed into cDNA using high-capacity cDNA transcription kit (Applied

Biosystems) with random hexameric primers as described in the manufacturer's protocol. The mRNA-expression was determined by Taqman real-time quantitative PCR (RT-qPCR, Applied Biosystems). cDNA was diluted 1/10 and then used for measurement of gene induction according to the manufacturer's instructions on an ABI Prism 7900HT Sequence Detection System using SDS2.2 software (Applied Biosystems). Gene expression was normalized to an internal control sample and to GAPDH. All murine primers were commercially available and measured with FAM TAMRA chemistry using the relative standard curve method. At the end of RT-qPCR cycle, dissociation curve analysis was performed to ascertain the amplification of a single PCR product.

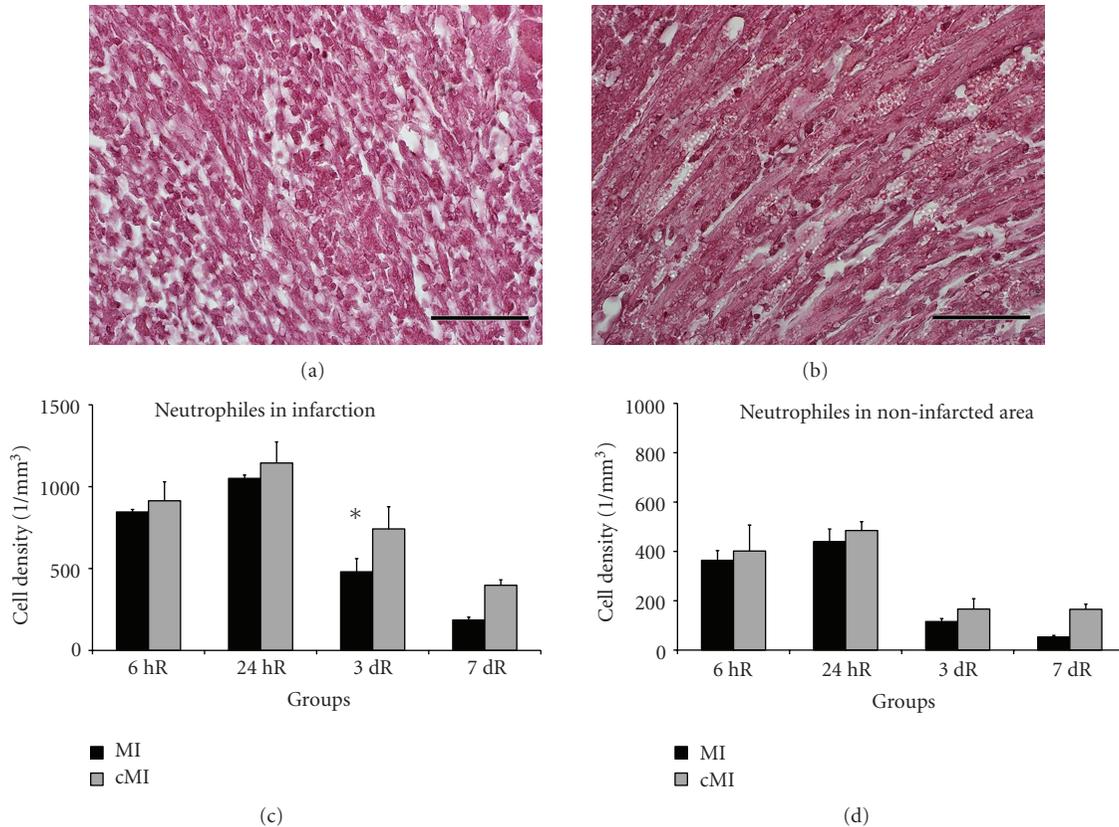


FIGURE 2: Neutrophils density in injured myocardium. Representative MCA 771 G staining for neutrophils (black-stained cells) in infarcted left ventricular wall (pink eosin counterstaining) 3 days after (a) reperfusion (MI) and (b) cryoinfarction (cMI). Comparison of neutrophils cell density in (c) infarcted area and (d) noninfarcted area in both models. Scale bar: 75  $\mu\text{m}$ ; \* $P < .05$ .

**2.6. Statistical Analysis.** All data are expressed as means  $\pm$  SEM. Two-tailed, unpaired Student's *t*-test was used to determine a significant difference between two groups. Multiple ANOVA was used to evaluate differences between three or more groups. Post hoc testing (Student-Newman-Keuls) was performed when appropriate. A  $P < .05$  was considered statistically significant.

### 3. Results

Histopathological features of cryoinfarction and reperfusion myocardial infarction in our model of cryoinfarction showed a transmural lesion with sharp infarction borders early after injury. The cryoinjury presented after 3 days with extensive cellular infiltration and partial granulation tissue formation starting at the periphery of infarction (Figure 1(a)). The central part of the cryoinjury at the copper probe application site contained numerous dead cardiomyocytes and cellular debris. The formation of loose granulation tissue was almost finished after 7 days (Figure 1(b)). The subsequent scar formation was largely completed after 14 days, but a substantial cellularity was still present in some parts of the cryoinjury whereas revascularization of large vessels was not observed (Figure 1(c); arrow). In contrast, the reperfusion of myocardial infarction in our closed-chest

model led to a nontransmural lesion. Complete formation of granulation tissue was found after 3 days (Figure 1(d)) and followed by a compacted scar formation with low cellular content after 7 days reperfusion (Figure 1(e)), as we published before [4]. The cryoinfarction showed large areas with loose collagen deposition after 7 days (Figure 1(f)) in contrast to compacted collagen-rich scar in reperfusion infarction at the same time point (Figure 1(g)). This difference in scar formation was further underlined by a vast number of myofibroblasts in cryoinfarction after 7 days (Figure 1(h)) whereas only few myofibroblasts were found in the reperfusion infarction (Figure 1(i)). The collagen was largely compacted in cryoinfarction after 14 days, but this was still associated with low myofibroblast persistence in the scar. The cryoinjury led to prolonged scar formation probably due to a longer time period needed for debris clearance, which could only progress from the periphery of the injury due to completely cryodamaged vasculature in the scar. In contrast, the reperfusion of myocardial infarction was associated with rapid scar formation due to an intact vascular network, which provided synchronous cell migration into the entire infarcted area.

**3.1. Comparison of Cellular Events between the Models.** Immunohistochemical staining of neutrophils revealed a

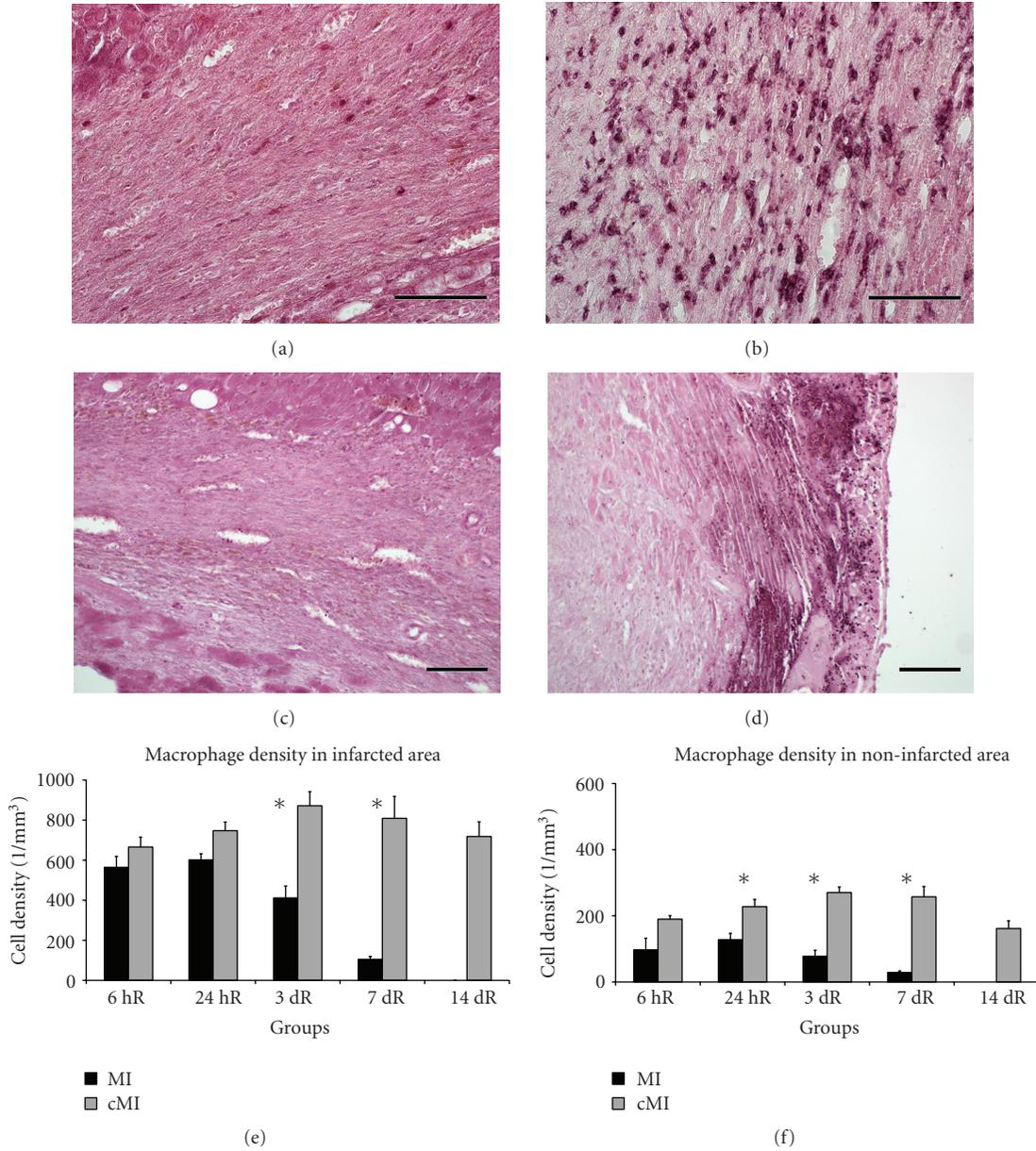


FIGURE 3: Macrophage density in cryoinfarction and reperfused infarction. Representative F4/80 staining for macrophages (black-stained cells) in left ventricular wall (pink eosin counterstaining) 7 days after (a) reperfused infarction (MI) and (b) cryoinfarction (cMI). Elastase staining showed lack of macrophage activity after 7 days in (c) MI in contrast to (d) ongoing activity (black staining) in cMI (pink eosin counterstaining). Comparison of macrophage cell density in (e) infarcted area and (f) noninfarcted area in both models. Scale bar (a)-(b): 75  $\mu\text{m}$ , (c)-(d): 100  $\mu\text{m}$ ; \* $P < .05$ .

strong myocardial infiltration early after the injury, suggesting a comparable myocardial debris clearance in both models. We found a significant difference in neutrophils density in infarcted area between the two models after 3 days (Figures 2(a) and 2(b)) and slightly longer neutrophils persistence in cryoinfarctions after 7 days (Figure 2(c)). We found no difference in cellular density of neutrophils in noninfarcted remote myocardium in both models (Figure 2(d)).

Macrophage staining showed a persistent, strong infiltration of cryoinjured myocardium after 7 days whereas only

few F4/80-positive cells were found after 7 days reperfusion of infarction, as previously published [4] (Figures 3(a) and 3(b)). We investigated macrophage activity using elastase staining after 7 days and found very strong signals in cryoinfarction, but no signals in reperfused infarction (Figures 3(c) and 3(d)). Interestingly, after 14 days, cryoinfarction showed much lower elastase activity, even though the total macrophage cell density was still high. The macrophage cell density data strongly support a short, rapid course of tissue remodeling after reperfusion of murine infarction,

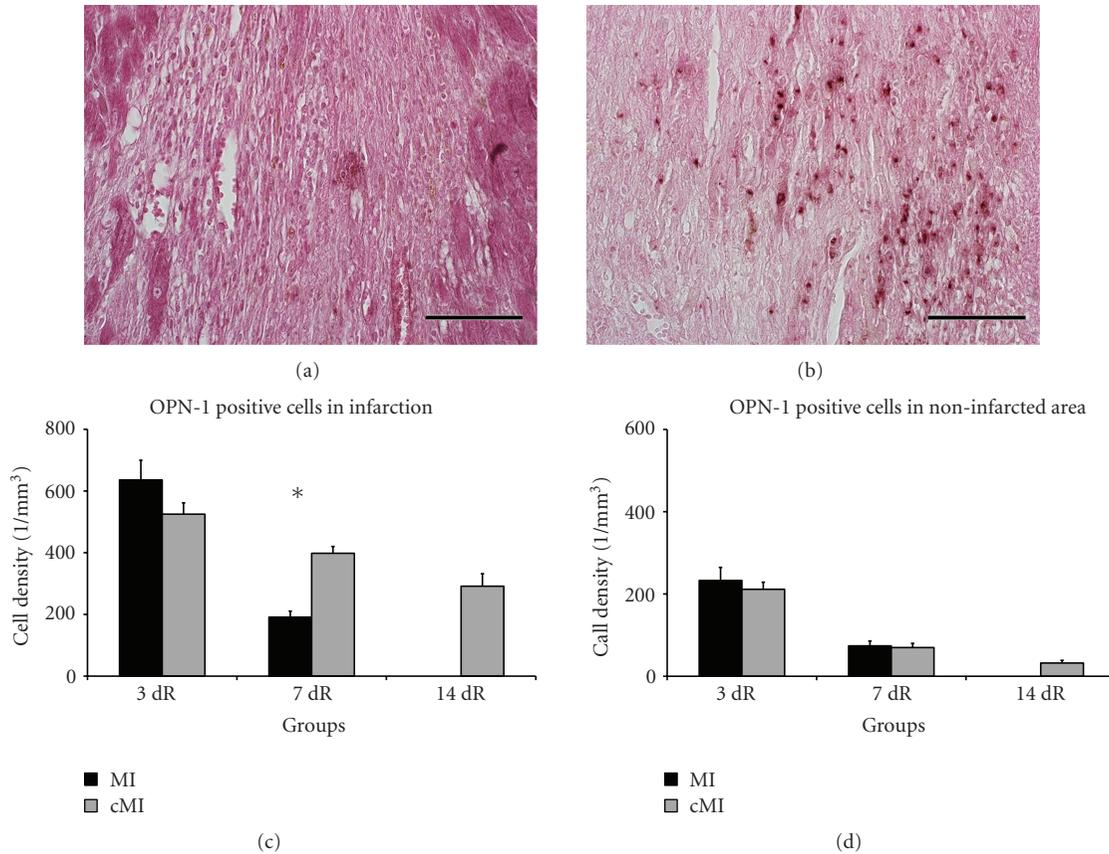


FIGURE 4: Osteopontin-1 in infarcted myocardium. Representative staining for macrophage maturation marker osteopontin-1 (black-stained cells) in left ventricular wall (eosin counterstaining) 7 days after (a) reperfusion (MI) and (b) cryoinfarction (cMI). Cell density of osteopontin-1-positive cells in (c) infarcted area and (d) noninfarcted area in both models. Scale bar: 75  $\mu\text{m}$ ; \* $P < .05$ .

in contrast to a persistent macrophage infiltration even 14 days after cryoinfarction (Figure 3(e)). To our surprise, macrophage density was also significantly higher in the remote, noncryoinjured myocardium, thus suggesting a dysfunctional development of the infarction border zone in this model (Figure 3(f)).

We further investigated the maturation of macrophages and found after 7 days a stronger infiltration of osteopontin-1-positive cells in cryoinfarcted hearts than in reperfusion myocardial infarction (Figures 4(a) and 4(b)). Evaluation of osteopontin-1-positive cell density revealed a prolonged, but functional maturation of macrophages (Figure 4(c)). The cell density of osteopontin-1-positive cells in the noninfarcted area was also comparable between the models (Figure 4(d)). Taken together, the course of cellular events involves a prolonged infiltration of cryoinfarction with inflammatory cells, thereby explaining the later development of a stable scar in this model. In contrast, a transient, short infiltration of reperfusion myocardial infarction with inflammatory cells is associated with a rapid resolution of myocardial remodeling.

**3.2. Differences in the Course of Molecular Events in Infarcted Myocardium.** The course of molecular events in cryoinfarction and reperfusion myocardial infarction was assessed using

mRNA-expression measurements of specific inflammatory and remodeling-related mediators. The myocardial damage leads to a rapid production of reactive oxygen species and induction of several scavenger enzymes, for example, heme oxygenase 1, glutathione peroxidase, and so forth. The expression of heme oxygenase 1 in reperfusion myocardial infarction accompanied the transient short inflammatory reaction with a peak induction after 24 h of reperfusion and a rapid downregulation thereafter (Figure 5(a)). The cryoinfarction led to a significantly different expression pattern with prolonged induction of heme oxygenase 1. The cytokine  $\text{TNF-}\alpha$ , the next downstream mediator in the postischemic inflammatory cascade, presented with a significantly stronger mRNA-induction early after reperfusion of infarction, which is probably triggering the rapid onset of inflammatory reaction (Figure 5(b)).  $\text{TNF-}\alpha$ -expression decreased thereafter in reperfusion myocardial infarction, in contrast to its maximal upregulation 3 days after cryoinfarction. We measured the induction of the anti-inflammatory cytokine IL-10 to investigate negative feedback regulation of proinflammatory response (Figure 5(c)) and found a comparable time course in both models with a significantly higher induction of it in cryoinfarction after 3 days. This probably reflects the prolonged proinflammatory response and delayed resolution of inflammation in cryoinfarction. Based

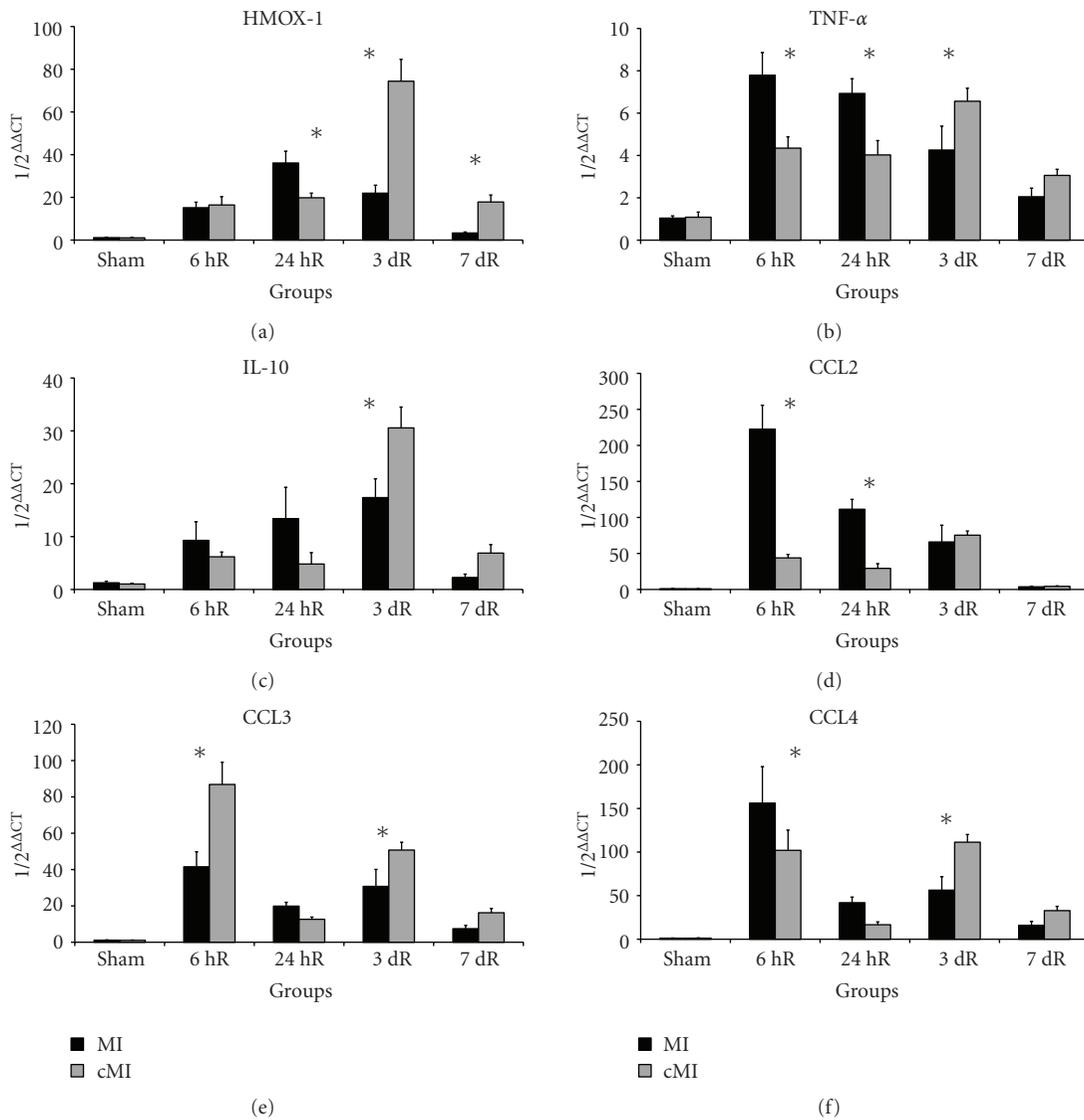


FIGURE 5: Expression profile of inflammatory mediators in cryoinfarction and reperfused infarction. RT-qPCR-analysis of mRNA-expression of (a) free radicals scavenger enzyme heme oxygenase 1, (b) proinflammatory cytokine TNF- $\alpha$ , (c) anti-inflammatory cytokine IL-10, (d) macrophage-related chemokine CCL2, (e) neutrophils-related chemokine CCL3 and (f) macrophage-related chemokine CCL4 in cryoinfarction (cMI) and reperfused infarction (MI). Data are normalized to respective shams and housekeeping gene GAPDH. \* $P < .05$ .

on our previous results regarding the role of chemokines in remodeling [4], we measured the expression of macrophage-related CCL2 and CCL4, as well as neutrophils-related CCL3. To our surprise, we found a significantly higher induction of CCL2 in reperfused infarction whereas cryoinfarction did not lead to its significant upregulation at a later time point (Figure 5(d)). In contrast, the mRNA-expression of CCL3 showed a significantly stronger early induction in cryoinfarction and a second maximum after 3 days when compared to reperfused infarction (Figure 5(e)). The chemokine CCL4 showed a comparable expression pattern between the models, but also significant differences at an early and later time point (Figure 5(f)). Taken together, the expression of free radical scavenger enzymes, cytokines and chemokines, is directly influencing the course of cellular

events in both models of infarction. Still, the expression of chemokines shows a different time course, probably due to their additional role in collagen deposition during advanced stages of remodeling.

Since osteopontin-1 is not only related to macrophage maturation but also is involved in extracellular matrix formation and remodeling, we measured its mRNA-expression and found an earlier peak after 24 hrs in reperfused infarction whereas in cryoinfarction its maximum was reached after 3 days (Figure 6(a)). Another marker of early remodeling, tenascin C, showed a significantly higher induction early after reperfusion of infarction, thus supporting the rapid granulation tissue formation (Figure 6(b)). Tenascin C-expression was significantly higher after 3 days in cryoinjured myocardium, thus mediating the prolonged granulation

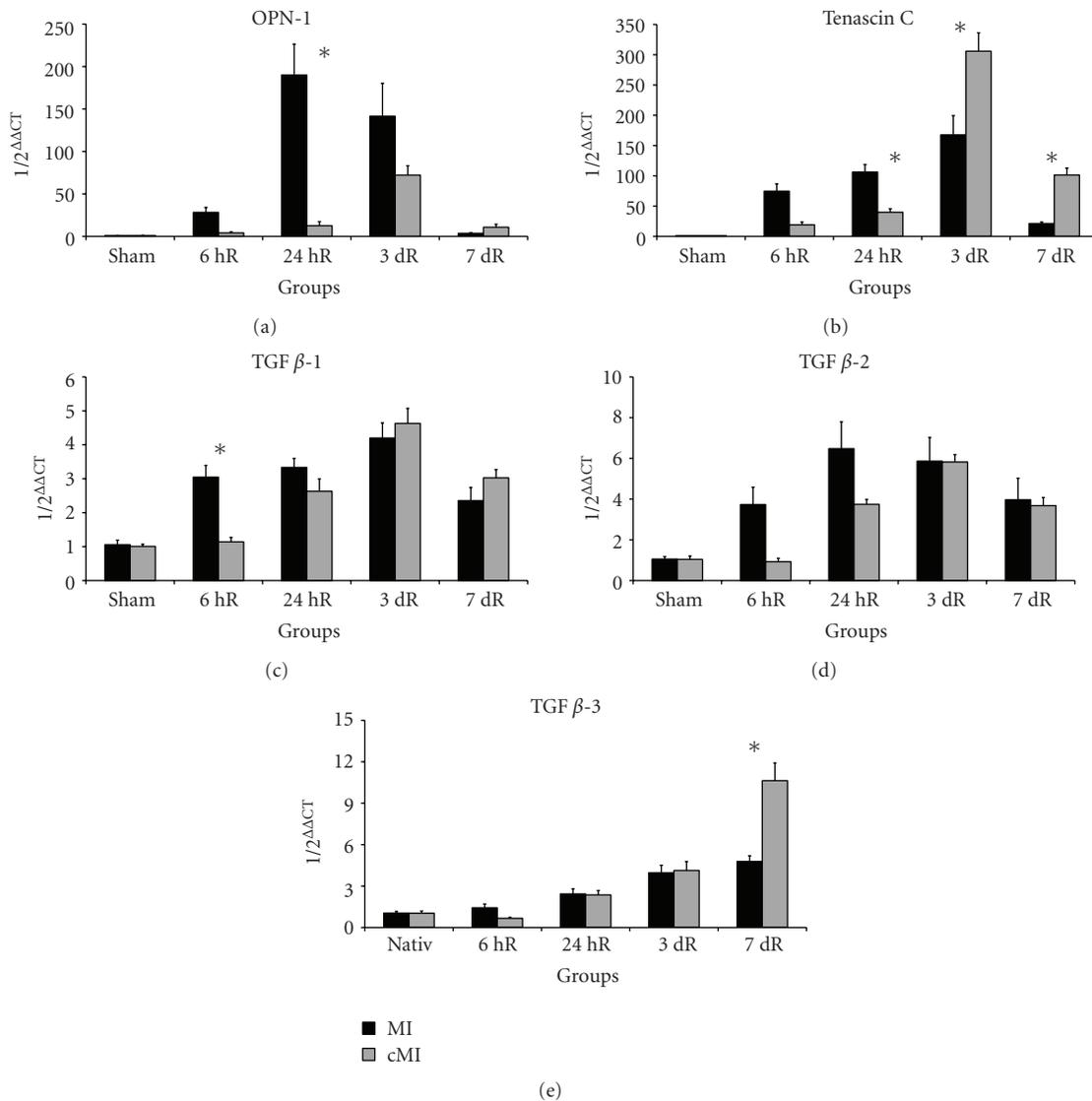


FIGURE 6: Expression profile of remodeling-related mediators in cryoinfarction and reperfused infarction. RT-qPCR-analysis of mRNA-expression of (a) macrophage maturation marker osteopontin-1, (b) early remodeling marker tenascin C, and isoforms of TGF- $\beta$ , an anti-inflammatory cytokine and remodeling mediator (c) TGF- $\beta$ -1, (d) TGF- $\beta$ -2, and (e) TGF- $\beta$ -3 in cryoinfarction (cMI) and reperfused infarction (MI). Data are normalized to respective shams and housekeeping gene GAPDH. \*  $P < .05$ .

tissue formation. We also investigated the mRNA-expression of TGF- $\beta$  and found a later induction of predominantly profibrotic acting TGF- $\beta$ -1 and - $\beta$ 2 isoforms (Figures 6(c) and 6(d)). Interestingly, we found a significantly higher induction of rather antifibrotic acting TGF- $\beta$ -3 isoform 7 days after cryoinfarction, thus probably representing a strong signal for the resolution of myocardial remodeling (Figure 6(e)). These data reveal specific mediators responsible for the prolonged granulation tissue formation and delayed remodeling process after cryoinfarction. Our data also confirm our previously published findings on induction of TGF- $\beta$  isoforms in reperfused infarction and give us additional novel insights in osteopontin-1- and tenascin C-expression in the reperfused infarction model.

#### 4. Discussion

Studies using large experimental models of myocardial infarction brought a detailed analysis of pathology and some insights into mechanisms involved in myocardial ischemia. Recent developments in transgenic mice and gene-targeting technologies provided tools for profound studies of the pathomechanisms and specific genes involved in cardiac repair. We developed a chronic, closed-chest murine model of myocardial ischemia followed by reperfusion, which leads to infarction with rapid scar formation [4]. This closed-chest model has minimized the influence of the initial surgery trauma and is, therefore, particularly useful for studies of inflammatory response and early remodeling. We used this

model in cellular therapy experiments and found a very poor engraftment of the i.v. injected whole bone marrow cells (unpublished observation) in contrast to a very good engraftment found in another model, that is, cryoinfarction [7]. Therefore, we assumed differences in myocardial remodeling and specifically in local microenvironment of injured myocardium. In order to investigate these differences, we compared the pathology and the course of cellular and molecular events during myocardial remodeling between the two models.

In this study, we confirmed our previous findings on rapid development of granulation tissue and the time course of mediators involved in transient inflammatory reaction, as well as in subsequent remodeling [3, 4]. We found a transient, strong increase in proinflammatory cytokines and chemokines leading to a rapid tissue infiltration with neutrophils and macrophages, which was also described in large animal models [12]. In addition, we observed a transient induction of heme oxygenase 1, which is caused by a massive production of reactive oxygen species during early reperfusion and precedes the cytokine release in the ischemic heart. Development of granulation tissue was associated with anti-inflammatory action of IL-10, with transient upregulation of early remodeling mediator tenascin C [13], and was followed by induction of macrophage maturation marker osteopontin-1 [14] and, as previously published, upregulation of TGF- $\beta$  isoforms [4]. These mediators lead to differentiation of myofibroblasts and subsequent collagen deposition, thus resulting in the stable scar formation 7 days after reperfusion of murine infarction [2]. The timely resolution of myocardial remodeling is also confirmed by a lack of macrophage elastase [15] and osteopontin-1 staining after 7 days reperfusion.

The pathology of cryoinfarction showed a similar course of events, but a longer duration of this process until formation of a stable myocardial scar is completed. The basic histology revealed that cryoinfarction leads to an early, strong cellular infiltration of the damaged myocardium, which slowly decreased to a lower level until 14 days postinjury. We observed a permanent damage and occlusion of the large vessels in cryoinjured myocardium, which mechanically prevented an evenly distributed cellular infiltration of the injured myocardium and thus rapid myocardial remodeling, as it is observed in reperfused infarction. In cryoinfarction, cell debris clearance is starting from the periphery, and the cells—macrophages and neutrophils—persist up to 14 days in the last area near the center of the epicardial copper probe application site. This seems to be one of the main factors influencing the pace of remodeling in the cryoinfarction model. Interestingly, we found a significantly prolonged macrophage infiltration of the noninjured area, which may be associated with a dysfunctional border zone formation and infarction limitation in this model. The concomitant appearance of elastase-producing macrophages and myofibroblasts 7 to 14 days postinjury represents an active myocardial remodeling process, which is further supported by not entirely compacted collagen in the scar. In consequence, the macrophage maturation marker osteopontin-1 shows prolonged cellularity in immunohistochemistry. This active

myocardial remodeling is probably responsible for a good engraftment of the implanted cells in the cryoinfarction model [7].

The mRNA data provide novel insights into the time course of the expression of several mediators involved in remodeling of cryoinfarcted myocardium. The later induction of cytokines and remodeling-related mediators is leading to the prolonged remodeling in this model. In particular, the relatively high expression of heme oxygenase 1 after 3 days represents persistent reactive oxygen species production from cell debris, while the high CCL2 and CCL4 expression characterize the inflammatory response associated with strong macrophage activity. The concomitant upregulation of anti-inflammatory IL-10 is acting towards resolution of the inflammatory reaction and formation of granulation tissue. The significantly higher expression of neutrophils-related chemokine CCL3 seems to reveal a strong migration stimulus into the damaged area, which is limited by the lack of intact vasculature as discussed above. Chemokines, particularly the CCL2, have also been associated with myofibroblasts activity and collagen production, which may provide additional explanation for its upregulation after 3 days in cryoinfarction [6]. Using transgenic mice, we described a crucial role for the chemokine CCL2 and macrophages in timely resolution of myocardial remodeling and preservation of myocardial function [5]. The later induction of tenascin C and osteopontin-1 as well as the TGF- $\beta$  isoforms represent the prolonged extracellular matrix production and collagen deposition in myocardial remodeling. TGF- $\beta$  itself has also been associated with the resolution of inflammatory response [16].

This study shows substantial differences in local microenvironment and cellular and molecular events between the models of cryoinfarction and reperfused infarction. In a comparison between murine models of cryoinfarction and coronary ligation without reperfusion, a modest adverse remodeling was postulated for the cryoinfarction, and the cryoinfarction was suggested as a representative model for myocardial infarction encountered in clinical practice [17]. We do not completely share this opinion, since the main goal in treatment of acute infarction is early revascularization with reperfusion. But for the experimental study purpose, the cryoinfarction indeed offers a very reproducible area at risk and infarct size whereas this is a major weakness of the reperfused infarction model due to the anatomical variability of coronary arteries. The prolonged myocardial remodeling seems to be beneficial in cell transplantation studies, since the cellular engraftment after direct application into injury is very good [9]. Our data provide novel insights into expression of cytokines and chemokines in cryoinfarction, where persistent proinflammatory milieu may be favorable for the cell engraftment. On the other side, the rapid resolution of inflammatory response in reperfused infarction may minimize the time span of suitable local environment for cell engraftment, but this concept still has to be further investigated. In respect to the ongoing clinical trials [18], the reperfused infarction model could be seen as a more relevant model, but it has not been widely used yet.

Our study has two major limitations. First, the findings are mainly of experimental interest since the model of reperfused myocardial infarction is comparable to a clinical situation in patients presenting with acute infarction, but it seems to be less favorable for the cell engraftment studies. On the other hand, the cryoinfarction model provides good conditions for cell engraftment and it is very reproducible, but of a limited value to the clinical practice. Second, we did not measure infarct size and one could argue that a difference in infarct size may explain the observed differences in pathological and molecular events between the two models. Based on previously published work, the model of reperfused infarction [4, 5] affects mostly a larger portion of left ventricle than the cryoinfarction [6], but the reperfusion still leads to a faster resolution of inflammation and scar formation than the cryoinjury. Therefore, the infarct size does not reflect the quality and pace of myocardial remodeling, and we did not find it to be necessary for explanation of the findings in this study.

## 5. Conclusions

In conclusion, the cryoinfarction is associated with prolonged inflammatory response leading to a postponed granulation tissue formation and scar development, when compared to the reperfused myocardial infarction. Several inflammatory mediators and remodeling factors are involved in this process, and they all contribute to a specific, dynamic local environment in ischemic myocardium. These substantial differences in remodeling may affect and even be favorable for cellular engraftment and should therefore be considered in cell therapy studies.

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