Published in final edited form as:

Semin Cell Dev Biol. 2016 October ; 58: 34–40. doi:10.1016/j.semcdb.2016.04.011.

Recent advancements in understanding endogenous heart regeneration—insights from adult zebrafish and neonatal mice

Nicole Rubin^{a,b,1}, Michael R. Harrison^{a,b,2}, Michael Krainock^{a,b,2}, Richard Kim^{a,b}, and Ching-Ling Lien^{a,b,c,*}

^aHeart Institute and Program of Developmental Biology and Regenerative Medicine, The Saban Research Institute of Children's Hospital Los Angeles, United States

^bDivision of Cardiothoracic Surgery, Department of Surgery, Keck School of Medicine, University of Southern California, United States

^cDepartment of Biochemistry & Molecular Biology, Keck School of Medicine, University of Southern California, United States

Abstract

Enhancing the endogenous regenerative capacity of the mammalian heart is a promising strategy that can lead to potential treatment of injured cardiac tissues. Studies on heart regeneration in zebrafish and neonatal mice have shown that cardiomyocyte proliferation is essential for replenishing myocardium. We will review recent advancements that have demonstrated the importance of Neuregulin 1/ErbB2 and innervation in regulating cardiomyocyte proliferation using both adult zebrafish and neonatal mouse heart regeneration models. Emerging findings suggest that different populations of macrophages and inflammation might contribute to regenerative versus fibrotic responses. Finally, we will discuss variation in the severity of the cardiac injury and size of the wound, which may explain the range of outcomes observed in different injury models.

Keywords

Zebrafish; Neonatal mouse; Heart regeneration

1. Introduction

Healthy cardiac function as essential to survival should not come as a surprise. Heart defects are the most common congenital defects [1–3]. Additionally, heart failure remains the major cause of morbidity and mortality in the westernized world [4]. From a human health perspective, research into the mechanisms of pathology and potential therapeutics for cardiovascular diseases requires urgent attention. Up until the end of the twentieth century, it was essentially dogma that the human heart has little in the way of regenerative potential. Clinical recovery following cardiac injury such as myocardial infarction is essentially a

*Corresponding author at: Heart Institute and Program of Developmental Biology and Regenerative Medicine, The Saban Research Institute of Children's Hospital Los Angeles, United States. clien@chla.usc.edu (C.-L. Lien).

¹Current address: Department of Cardiology, University of California, San Diego.

²These authors contributed equally to this work.

matter of scar formation and adaptation to the residual diminished capacity of the combination of remaining cardiomyocytes and fibrin scar. To this day, therapeutic regimens such as ACE (angiotensin converting enzyme) inhibitors, Beta-blockers and anti-platelet agents are primarily focused on improving our ability to handle the consequences of injury and preventing additional complications rather than being focused directly on addressing the processes involved in injury resolution and the restoration of functional cardiac architecture [5,6].

Regenerative medicine-based strategies have great potential in curing degenerative diseases rather than simply ameliorating the symptoms caused by these diseases. Recent regenerative strategies for myocardial repair have largely focused on the application of exogenous cells to the damaged heart and different progenitor populations that may exist and contribute to the process of recovery from injury [7–10]. However, several factors combined to open a new direction in cardiac research that highlighted the endogenous regenerative potential of the human heart and began to offer insights that fundamentally changed the way that we looked at myocardial injury, offering hope for new exciting therapies and treatment options. Our interest in endogenous myocardial regeneration, as well as our current understanding of the process, stems largely from lessons learned from the zebrafish heart. The zebrafish demonstrate a remarkable ability to repair its myocardium following a variety of forms of injury. More recently, the neonatal mammalian heart has been shown to possess a similar potential for endogenous repair. Lastly, several publications conclusively demonstrated that cardiomyocyte self-renewal does in fact occur in human hearts, albeit at a low level that diminishes with age [11–13].

In this review, we will discuss the development, similarities, and differences between adult zebrafish and neonatal mouse heart regeneration models. We will review recent discoveries of molecules, cells, and tissues that are essential for zebrafish and neonatal mice heart regeneration. Finally, we will discuss the role of inflammation and how differences in the extent of injury can potentially lead to the activation of different regenerative pathways.

2. Development of heart injury models to study cardiac regeneration in adult fish and neonatal mouse

Compared to mammals, zebrafish have a remarkable capacity to regenerate damaged tissues and organs [14,15]. The research on the regenerative capacity of zebrafish was initially focused on appendage regeneration using amputation of the caudal fin as a model [15–21]. To further study the molecular mechanisms of organ-specific regeneration of the heart, an injury model was developed in which 20% of heart ventricle is amputated/resected using surgical (iridectomy) scissors [22]. Surprisingly, zebrafish can survive this traumatic injury. A blood clot forms after resection of the heart apex and bleeding stops in a few seconds. After returning to the water for recovery, the zebrafish heart fully regenerates after 1–2 months [22,23].

Adult mammalian hearts fail to regenerate and undergo fibrosis during the process of heart repair after an injury. Heart hypertrophy and dilation may occur during this process, and this eventually can lead to deterioration of cardiac function [24–26]. A similar heart injury model

was developed to test whether neonatal mice possess regenerative capacity like adult zebrafish [27]. Since blood pressure in neonatal mice is higher than that of adult zebrafish [28,29], it is unlikely that the neonatal mouse could sustain a heart injury like ventricular resection and survive. However, neonatal mice can survive long hypothermia, which has been used to induce apnea and asystole to prevent excessive bleeding [27,30–32]. This anesthesia method is critical for the establishment of the ventricular resection model in neonatal mice.

3. Cardiomyocyte proliferation to replenish lost myocardium

The most critical event that occurs during zebrafish heart regeneration is the replenishment of lost cardiomyocytes that form an electrically coupled contractile syncytium. Using lineage tracing, it was found that newly regenerated cardiomyocytes arise from dedifferentiation and proliferation of the remaining cardiomyocytes [33–35]. The cardiomyocytes at the injury area lose their cell-cell attachments and form large intercellular spaces, concomitant with the disorganization of the actin and myosin cytoskeleton, sarcomere, and the Z-lines [33,34]. Phosphohistone H3 (pHH3) and proliferating cell nuclear antigen (PCNA) staining indicate that sarcomeric disassembly occurs in cardiomyocytes that have reentered the cell cycle, and accordingly 5-bromo-2'-deoxyuridine (BrdU) staining has further revealed that cardiomyocytes with disassembled sarcomeres resume DNA synthesis [33,34]. The finding of structural dedifferentiation in cardiomyocytes undergoing proliferation following myocardial injury suggests an alteration in gene expression. Using transgenic fish harboring reporter constructs, Kikuchi et al. demonstrated that zebrafish cardiomyocytes re-express the cardiogenic transcription factor Gata4 following myocardial injury. Interestingly, Gata4 expression is initially activated in the sub-epicardial compact layer of cardiomyocytes. These *gata4+* cardiomyocytes proliferate then populate the injured area of the heart ventricle, suggesting cardiomyocyte migration might play an important role in myocardial regeneration as well as cardiomyocyte proliferation [34]. Using a photoconvertible Kaede fluorescent reporter that can be used to mark specific cell lineages, it was demonstrated that cardiomyocyte migration is indeed essential for myocardial regeneration and that the Cxcl12a/Cxcr4b chemokine signaling axis is critical for this process [36]. It has not yet been determined if Cxcl12a/Cxcr4b signaling specifically regulates migration of *gata4+* cardiomyocytes. In addition to Gata4, Schindler and colleagues demonstrated that *hand2* (which encodes the Hand2 transcription factor) is expressed in regenerating zebrafish hearts and its overexpression augments cardiomyocyte proliferation in the injured zebrafish heart [37]. This result further supports the notion that re-expression of cardiogenic factors is essential for cardiomyocyte dedifferentiation and proliferation.

Similar to the adult zebrafish, cardiomyocyte proliferation accounts for the regenerative capacity of neonatal mouse hearts after ventricular resection [27]. However, this capacity of neonatal mouse hearts is lost after postnatal day 7 (P7) when cardiomyocytes stop proliferating and start to undergo binucleation and hypertrophy [27]. Many factors and molecules have been shown to play important roles in regulating cardiomyocyte proliferation during zebrafish heart regeneration [reviewed in [38,39]]; however, it remains to be determined if these factors and genes are important for mammalian cardiomyocyte

proliferation. One factor that is essential in regulating cardiomyocyte proliferation in both zebrafish and mice is Neuregulin 1 (Nrg1). *nrg1* is highly induced in perivascular cells after heart injury in zebrafish [40]. Blocking the Nrg1 co-receptor ErbB2 using the chemical inhibitor AG1478 inhibits cardiomyocyte proliferation during zebrafish heart regeneration. Moreover, cardiomyocytes specifically overexpressing Nrg1 using inducible transgenes is sufficient to induce proliferation of ventricular wall cardiomyocytes even without inducing cardiac injury [40]. In addition to a hyperplasia response, Nrg1 overexpression in cardiomyocytes is sufficient to initiate a heart regeneration program, including expression of *gata4*, activation of epicardium, and growth of new coronary vessels, even in the absence of cardiac injury [40]. Among all the genes and factors tested in zebrafish heart regeneration, Nrg1 seems to be the most potent factor that is both necessary and sufficient for cardiomyocyte proliferation and myocardial regeneration. One major difference between adult zebrafish and mammalian hearts in cardiomyocyte composition is that most cardiomyocytes in adult zebrafish are mono-nucleated while cardiomyocytes in adult mammalian hearts are mainly bi-or multi-nucleated. Similar to adult zebrafish hearts, neonatal hearts have mainly mononucleated cardiomyocytes at postnatal day 1 (P1). Nrg1 can also stimulate proliferation of neonatal cardiomyocytes efficiently [41,42]. While Nrg1 is not a potent inducer of bi-nucleated cardiomyocyte proliferation, a small population of mononucleated cardiomyocytes that responds to Nrg1 treatment has been identified in adult mouse hearts [41]. Nrg1 signals through ErbB4 and its co-receptor ErbB2. Deleting ErbB4 receptor in cardiomyocyte-specific knockout mice inhibits Nrg1 induced cardiomyocyte proliferation [40,41]. Interestingly, expression of ErbB2 at both the RNA and protein levels is significantly decreased at P7 compared to P1 [43]. This could potentially explain why cardiomyocytes fail to respond to Nrg1 stimulation of adult mouse hearts [44]. Overexpression of a constitutively active ErbB2 is sufficient to induce cardiomyocyte proliferation in adult mouse hearts [43]. The constitutively active ErbB2 can not only induce complete cell division of mononucleated cardiomyocytes but can also induce the formation of two mononucleated daughter cells from bi-nucleated cardiomyocytes [43]. Altogether, these results suggest that Nrg1 and its receptors ErbB4 and ErbB2 are essential for zebrafish and neonatal and adult mammalian cardiomyocyte proliferation and myocardial regeneration, and that intrinsic properties of the cardiomyocytes determine their responsiveness to growth factor stimulation [41–43,45,46].

Nerves, in particular cholinergic nerves (those that release the neurotransmitter acetylcholine), have been known to play a crucial role in many regenerative responses of a number of species [47]. Blocking acetylcholine transmission pharmacologically in both adult zebrafish and neonatal mice following apical resection results in a significant decrease in cardiomyocyte proliferation [48]. A similar proliferation deficit is observed with cardiac hypoinnervation in transgenic zebrafish and vagotomy (mechanical disruption of the parasympathetic left vagus nerve) in neonatal mice, suggesting that nerves directly support this proliferation. Further, these models both show significant scarring after 30 and 21 days of treatment respectively, and in the case of neonatal mouse vagotomy a consistent increase in mortality is observed. Significant scarring after 21 days is also observed with specific chemical denervation of adrenergic (sympathetic) nerves in the neonatal mouse heart [49]. This suggests the sympathetic (adrenergic) and parasympathetic (cholinergic) nerves could

play non-redundant roles in supporting regeneration or that compromising one can affect the ability of the other to carry out this function. Vagotomy results in a decrease in the cell cycle regulators cyclin D2 (Ccn2) and cyclin dependent kinase 4 (Cdk4), and the growth factors Nrg1 and nerve growth factor (Ngf) [48]. When these two growth factors were exogenously supplied to regenerating hearts that had undergone vagotomy, the deficits observed in proliferation and regeneration appeared to be alleviated [48]. Although clearly vital for the heart to mount a successful regenerative response to injury, it is not clear if these nerves provide such mitogens directly or stimulate their expression. In addition to the stimulation of proliferation, there is a weaker inflammatory response to injury in hearts that have undergone vagotomy [48]. As discussed below, loss or changes to this inflammatory response also has implications for the heart's ability to regenerate following injury. It will be interesting to further elucidate the role of the nervous system in support of these two regenerative responses and their potential cooperation during effective heart regeneration.

In addition to cardiomyocyte proliferation, revascularization of regenerating hearts occurs naturally for both adult zebrafish and neonatal mouse hearts after resection of heart apex [27,35,38,50]. The response to injury is also accompanied by the activation of epicardium (i.e. expression of embryonic markers) in both zebrafish and neonatal mouse hearts [27,35,38,50]. This activated epicardium has been shown to contribute to perivascular cells during zebrafish heart regeneration [51] and is a source of signaling factors that support the regenerative response [52]. These aspects are not the focus of discussion in this review, but are discussed at length in other review articles [35,38,39,53].

4. Cryoinjury as an alternative model

Although heart amputation/ventricular resection has been established as a standard procedure to study how heart regeneration occurs to replace lost cardiac tissues, this model does not mimic the pathophysiology of myocardial infarction. A more clinically relevant cryoinjury model has been established to model myocardial infarction in zebrafish and to allow for the study heart regeneration in this context [54–56]. A cryoprobe (either a 0.3 mm copper or 0.8 mm stainless steel filament) pre-chilled in liquid nitrogen is placed on the heart apex to damage the heart by freezing and thawing the cells, which leads to cell death [54,55,57]. Unlike the amputation model in which healthy cardiac tissues are removed, dead tissues remain in the injured hearts after cryoinjury and might trigger more robust or longer inflammatory responses [35,55]. Similar to myocardial infarction in mammalian hearts, this prolonged inflammation leads to fibrosis and collagen scar deposition. Yet, in contrast to the injured mammalian heart, the collagen scar is later resolved and the zebrafish heart regenerates between 60 and 90 days post cryoinjury [54,55]. Interestingly, differential scarring and collagen deposition has been reported by different groups using different methods to create cryoinjury in zebrafish hearts. While collagen scars are observed after cryoinjury induced by metal cryoprobes [54,55], minimal collagen scarring occurred during the regeneration process when cryoinjury was induced by placing a small piece of dry ice on the heart ventricle; instead, mainly fibrin clots formed [56]. Comparing these reports led us to hypothesize that different severities of cryoinjury induce different regenerative responses. In fact, we observed large variations in the regenerative/scarring responses of zebrafish hearts to cryoinjury even when different researchers operated with the same metal

cryoprobe. This variation we observed raises the importance in carefully evaluating and comparing time course and data from different groups (Table 1).

5. Role of inflammation in heart regeneration and fibrosis

Unlike cardiomyocyte proliferation, post-injury inflammation plays a much more complicated role in the regeneration process. Inflammation cannot be easily dismissed as a detrimental aspect of cardiac injury, as has been the case with using steroidal [58] and non-steroidal [59] anti-inflammatories as clinical treatments post-MI. Mechanistic studies in animal models have demonstrated inflammation as an indispensable process to repair [60,61] and regeneration of the heart after injury [62–64]. Here we discuss the roles of inflammation in regenerative hearts of zebrafish and neonatal mouse that have been elucidated thus far.

5.1. Role of inflammatory response

Gene expression profiling of regenerating zebrafish hearts after ventricular resection revealed upregulation of inflammation and injury related genes in the regenerative process [65]. Injury-related recruitment of platelets to the injury area in the initial blood clot can provide growth factors, such as platelet-derived growth factor (Pdgf), which regulates epicardial functions and new vessel formation [66]. However, how the local injury/inflammatory response affects heart regeneration and reactivation of a developmental program in zebrafish has not been studied until recently. NF- κ B signaling is involved in a wide range of processes, although it has been mostly studied in inflammatory cells as a stress response and in mediating TNF family cytokine signaling [67–71]. Interestingly, it has been demonstrated that NF- κ B signaling is activated in cardiac muscles adjacent to the injury area during zebrafish heart regeneration [72]. Inhibiting NF- κ B signaling by overexpressing a super repressor I κ B in cardiomyocytes causes decreased cardiomyocyte proliferation and failure in zebrafish heart regeneration [72]. Whether NF- κ B affects inflammatory response during zebrafish heart regeneration remains to be determined. Furthermore, NF- κ B signaling is also activated in mammalian hearts after myocardial infarction [73,74]. Comparing how zebrafish and mammalian hearts respond to NF- κ B signaling after heart injury might provide insights in how endogenous regenerative capacity might be enhanced.

Another issue that can complicate the comparison between neonatal and adult mammalian hearts and zebrafish hearts is that paradoxical roles of inflammation can either lead to deterioration or promote organ repair and regeneration [64,75–77]. An inflammatory response occurs in response to injury of the non-regenerative adult mammalian heart and this has been examined with regard to the injury, repair, and remodeling processes [reviewed in [60,61]]. However, different neonatal mouse heart injury models also demonstrate that inflammation occurs during the process of regeneration [62–64]. Age-dependent macrophage/monocyte responses were identified in neonatal mouse heart in response to injury; macrophages at the age of P1 promote regeneration while macrophages from older mice could only clear cellular debris and secrete soluble factors that promoted fibrosis [62]. Interestingly, even cardiomyocyte proliferation is crucial for neonatal mouse heart regeneration; P1 macrophages regulates angiogenesis instead of cardiomyocyte proliferation

that are required for heart regeneration in a neonatal myocardial infarction model [62]. The macrophages also showed a differential pattern of distribution; in the P1 mice with injury, macrophages were more abundant and uniformly distributed, whereas there were fewer macrophages in P14 mice with injury and they were mainly localized to the ischemic zone [62]. Furthermore, macrophages in neonatal mouse hearts have different polarization and transcriptional profiles when compared to older mice [62]. It is unclear whether the signals that recruit these macrophages are different at P1 and P14 or if different subpopulations of macrophages respond differently to similar recruiting signals.

This pattern of differential macrophage populations in injured non-regenerative heart is consistent in other injury models, such as a diphtheria toxin (DT) induced ablation of cardiomyocytes [64]. In this ablation model, a population of resident cardiac macrophages expands and promotes heart regeneration via cardiomyocyte proliferation and angiogenesis [64]. Genetic lineage tracing revealed that these macrophages promoting neonatal mouse heart regeneration with minimal inflammation are embryonically-derived but not derived from definitive hematopoiesis [64]. Using another injury model induced by apical microinjection of immunogenic zymosan A particle (ZA), acute inflammation was also shown to induce cardiomyocyte proliferation and neonatal mouse heart regeneration [63]. Furthermore, immunosuppression inhibits neonatal heart regeneration. Induction of the inflammatory genes interleukin 6 (*il6*), interleukin 1b (*il1b*), and chemokine C-C motif ligand 3 (*ccl3*) are observed in this injury model and are required for neonatal heart regeneration [63]. In zebrafish heart, cytokine signaling transducer and activator of transcription 3 (Stat 3) and downstream mediators are also induced early after tissue damage. Stat3 regulates expression of Relaxin 3a (Rln3a), which stimulates cardiomyocyte proliferation [78].

The neonatal inflammatory process is beneficial and regenerative by influencing angiogenesis and not participating in adverse tissue remodeling [64]. Furthermore, inhibiting monocyte recruitment after adult heart injury is sufficient to preserve the resident embryonically-derived macrophage lineage [64]. Using these results as a guide, non-regenerative adult heart repair could be improved by promoting macrophage recruitment over that of monocytes.

5.2. Role of ECM and fibrosis

The extracellular matrix may be the most under-appreciated participant in the repair and regeneration process. The extracellular matrix plays an important role in permitting, and at times orchestrating, cellular processes necessary for repair and regeneration. This acellular scaffold not only physically supports cells but is also dotted with growth factors or cell signals to guide migratory cells. For instance, in the amputation model the initial formation of a blood clot followed by a fibrin clot provides an extracellular matrix that closes the lumen of the ventricle [22] while providing a scaffold for migrating cardiac cells to repopulate and regenerate complete myocardium [36]. As demonstrated in ex vivo assays, the epicardial cells of the regenerating heart are migratory on fibrin [79]. In vivo the zebrafish epicardium also expresses migratory markers; it proliferates and expands to cover the fibrin clot [50,66]. One major ECM component in regenerating zebrafish hearts

deposited by epicardial cells is fibronectin; loss of function in fibronectin disrupts heart regeneration [80].

One of the main barriers for patients of myocardial infarction is the scarring, or persistence of fibrosis, in the myocardium. After surviving the initial ischemic injury, the resulting fibrosis not only causes morbidity due to replacement of functional cardiomyocytes, but it can also lead to mortality as a non-contractile wall with reduced thickness that is vulnerable to rupture remains. In the adult mammalian heart, the deposition of a collagenous extracellular matrix is irreversible, at least as of yet. Conversely, heart regeneration in zebrafish [54,57] and neonatal mice can progress beyond scar deposition to scar resolution, and thus favors functional cardiomyocyte replacement. Cryoinjury to the zebrafish heart initially results in collagen deposition to replace apoptotic and necrotic cells, but eventually the collagen scar is resolved and replaced by cardiomyocytes resulting in a regenerated heart [54,57]. This transient scar formation is mediated by transforming growth factor (TGF)-beta/Activin-Alk5 signaling and is actually necessary for the regeneration process to proceed. Inhibition of TGF-beta/Activin-Alk5 signaling results in decreased cardiomyocyte proliferation and a bulging ventricle phenotype [81]. The capacity to resolve the fibrotic tissue constitutes a significant step in heart regeneration.

6. Regenerative responses to different severities of injuries

The apical resection model in zebrafish has been a reproducible model for understanding the regenerative process in an adult vertebrate heart. In contrast, differences in the capacity for complete regeneration of neonatal mouse hearts after apical resection have been reported by different laboratories [82–87]. Andersen et al. showed that apically resected myocardium is not regenerated, despite claims of similar amounts of tissue (or less) removed (~8.5% versus ~15% in Porrello et al.). Bryant et al. systematically evaluated variables for the apical resection surgery, and found that variability in the amount resected tissue influenced the extent of fibrosis, yet both a moderate tissue loss of 10% relative to heart weight and a large tissue loss of 20% resulted in fibrosis. This study suggests that the discrepancy from different laboratories studying neonatal mouse heart regeneration is most likely a result of variation of the severity in heart injury created by ventricular resections. A simple explanation is that regeneration of neonatal mouse hearts is limited to a window (P1–P7) during which cardiomyocytes still can divide before they undergo binucleation and hypertrophy, which are normal processes during postnatal cardiac development. The bigger the heart injury is, the longer it might take to fully regenerate. When the injury is large and requires more than 7 days to regenerate, collagen residue might remain to fill in spaces that are not fully replaced by regenerated cardiomyocytes by this time.

Compared to ventricular resection, it is easier to control the severity of injury using cryoinjury. The regenerative neonatal (P1) heart undergoes minimal adverse remodeling, compared to the adult mouse heart [64]. If the extracellular matrix in the injury site progresses extensively, it may exceed the capacity to be resolved completely, similar to what was observed in zebrafish after cryoinjury. Indeed, the cryoinjury model suggests a spatial limitation to the regenerative capacity of the neonatal heart [88]. By increasing the depth and extent of injured tissue, the injury and repair response can be modulated in severity. For

instance, the expression of plasminogen activator inhibitor protein 1 (PAI-1) is induced after cryoinjury, but only in the transmural (full-wall thickness) injury does it persist after 7 days post-injury [88]. High PAI-1 levels can lead to decreased collagen degradation and to the maintenance of extensive fibrosis in the more severe cryoinjury. We did observe that neonatal hearts with transmural injury had increased fibrosis compared to hearts with partial-wall thickness injuries (Table 1); thus, the more extensive injury may exceed the limited ability of the hearts to resolve the collagenous extracellular matrix, resulting in a large persistent scar [88]. Examination of the injury severity reveals that the responses that occur can be measurably different [88,89] in so much as that they could contribute to the repair versus regeneration outcome. Therefore, the variability in the injury type and severity needs to be carefully evaluated and a standard should be established for reporting the results.

7. Conclusions and perspectives

Studies on endogenous cardiac regeneration started with the zebrafish apical amputation as an adult vertebrate model of complete heart regeneration [22]. Subsequently this has expanded to include an adult zebrafish cryoinjury model and genetic ablation model [54,57,90]. A decade later, transient regenerative potential was shown for neonatal mouse heart [27]. Like the zebrafish, the neonatal mouse cardiac injury models expanded from apical resection to include cryoinjury [42,84,88], coronary artery ligation [91], diphtheriatoxin induction [64], and zymosan A bead injection [63].

As we have highlighted in this review, major mechanisms shared by both the zebrafish and the neonatal mouse suggest that there are potential targets for initiating the regenerative response in the injured adult human heart. Promoting cardiomyocyte proliferation by modulating growth factors (such as Neuregulin), innervation and cell signaling pathways might be essential to replenish damaged or lost cardiac tissue for a regeneration response. These models have also demonstrated that collagen deposition is not contrary to regeneration since zebrafish heart can regenerate after scar resolution (although extent of injury can prove a limitation to complete regeneration). The standard anti-inflammatory therapies can be improved to harness the balance of inflammation's positive effects with its detrimental effects, as these models have demonstrated it is essential and there are subtle differences between the regenerative heart versus the non-regenerative heart. With the novelty of these two models and these recent observations into regenerating hearts, there remains further insight to be gathered into these specific mechanisms and the as-yet undiscovered mechanisms.

7.1. Applications and bottlenecks for application

The zebrafish models have been effective in demonstrating varying modes of injury to cardiac cells, as well as how the organ and its various cellular populations respond in order to regenerate lost tissue. Fortunately the void of a more complex mammalian model was filled with the neonatal mouse. In a short amount of time, zebrafish and neonatal mice have been used to model different aspects of cardiac regeneration, including many of the basic cellular mechanisms and whole organ processes. The roles of different cell populations in the regeneration process have emerged. For instance, the epicardium can undergo an

epithelial-to-mesenchymal transition (EMT) to contribute to perivascular cells during the regeneration process. Taken together, many of the processes of regeneration recapitulate embryonic development.

Comparisons with zebrafish have also revealed some of the insufficiencies in mammalian hearts that prevent complete regeneration, such as a lack of cardiomyocyte proliferation. This is one of the major therapeutic bottlenecks that are necessary to overcome to accomplish at least enhanced repair. Stimulating cardiomyocyte proliferation may be as simple as providing exogenous growth factors or pharmacological stimulators of pathways defined thus far in these regenerative models. However, further investigation into the feasibility for therapeutic intervention is needed. For instance, it is unknown whether essential growth factor receptors are still expressed in adult hearts.

7.2. Open questions

A major void in the research thus far is the lack of comparison in physiology between a regenerative heart and non-regenerative heart. Although the zebrafish has a two-chambered heart compared to the four-chambered mammalian heart, it only has a slightly increased heart rate compared to the human heart. On the other hand, at birth mice have a heart rate that is at least 3 times of the human, with the heart rate doubling as the animals reach adulthood. The significance of changing the physiology and output of the heart could be highly relevant to the regenerative capacity due to the physical demands arising from physiological function that might compete with or counteract regenerative needs. The extracellular changes associated with the demands of higher output may also be hindering the ability of new cardiac cells to develop and repopulate injured tissue and the resolution of scar. These types of future experiments, as well as continued studies on cellular and molecular mechanisms underlying various aspects of regeneration (e.g., the role of different inflammatory cell populations) will hopefully lead to improved therapeutic strategies for heart disease, which remains the leading cause of mortality worldwide.

Acknowledgments

We thank M. Chao for critical reading of the manuscript. This work was supported by National Heart, Lung and Blood Institute Grant (R01HL096121 to C.-L. L.) and a Research Career Development Fellowship from the Saban Research Institute (to M.H.).

References

1. Postma AV, Bezzina CR, Christoffels VM. Genetics of congenital heart disease: the contribution of the noncoding regulatory genome. *J Hum Genet.* 2016; 61:13–19. [PubMed: 26223183]
2. van der Bom T, Bouma BJ, Meijboom FJ, Zwinderman AH, Mulder BJ. The prevalence of adult congenital heart disease, results from a systematic review and evidence based calculation. *Am Heart J.* 2012; 164:568–575. [PubMed: 23067916]
3. van der Bom T, Zomer AC, Zwinderman AH, Meijboom FJ, Bouma BJ, Mulder BJ. The changing epidemiology of congenital heart disease. *Nat Rev Cardiol.* 2011; 8:50–60. [PubMed: 21045784]
4. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M. Executive summary: heart disease and stroke statistics—2016 update: a report from the american heart association. *Circulation.* 2016; 133:447–454. [PubMed: 26811276]
5. Najafi A, Sequeira V, Kuster DW, van der Velden J. Beta-adrenergic receptor signalling and its functional consequences in the diseased heart. *Eur J Clin Invest.* 2016

6. Szema AM, Dang S, Li JC. Emerging novel therapies for heart failure. *Clin Med Insights Cardiol.* 2015; 9:57–64. [PubMed: 26512208]
7. Behfar A, Crespo-Diaz R, Terzic A, Gersh BJ. Cell therapy for cardiac repair—lessons from clinical trials. *Nat Rev Cardiol.* 2014; 11:232–246. [PubMed: 24594893]
8. Laflamme MA, Murry CE. Heart regeneration. *Nature.* 2011; 473:326–335. [PubMed: 21593865]
9. Marban E, Cingolani E. Heart to heart: cardiospheres for myocardial regeneration. *Heart Rhythm.* 2012; 9:1727–1731. [PubMed: 22813578]
10. Segers VF, Lee RT. Stem-cell therapy for cardiac disease. *Nature.* 2008; 451:937–942. [PubMed: 18288183]
11. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, et al. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med.* 2001; 344:1750–1757. [PubMed: 11396441]
12. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, et al. Evidence for cardiomyocyte renewal in humans. *Science.* 2009; 324:98–102. [PubMed: 19342590]
13. Molloy M, Bersell K, Walsh S, Savla J, Das LT, Park SY, et al. Cardiomyocyte proliferation contributes to heart growth in young humans. *Proc Natl Acad Sci U S A.* 2013; 110:1446–1451. [PubMed: 23302686]
14. Poss KD. Advances in understanding tissue regenerative capacity and mechanisms in animals. *Nat Rev Genet.* 2010; 11:710–722. [PubMed: 20838411]
15. Poss KD, Keating MT, Nechiporuk A. Tales of regeneration in zebrafish. *Dev Dyn.* 2003; 226:202–210. [PubMed: 12557199]
16. Akimenko MA, Johnson SL, Westerfield M, Ekker M. Differential induction of four *msx* homeobox genes during fin development and regeneration in zebrafish. *Development.* 1995; 121:347–357. [PubMed: 7768177]
17. Ferretti P, Geraudie J. Retinoic acid-induced cell death in the wound epidermis of regenerating zebrafish fins. *Dev Dyn.* 1995; 202:271–283. [PubMed: 7780176]
18. Geraudie J, Monnot MJ, Brulfert A, Ferretti P. Caudal fin regeneration in wild type and long-fin mutant zebrafish is affected by retinoic acid. *Int J Dev Biol.* 1995; 39:373–381. [PubMed: 7669548]
19. Johnson SL, Bennett P. Growth control in the ontogenetic and regenerating zebrafish fin. *Methods Cell Biol.* 1999; 59:301–311. [PubMed: 9891366]
20. Johnson SL, Weston JA. Temperature-sensitive mutations that cause stage-specific defects in zebrafish fin regeneration. *Genetics.* 1995; 141:1583–1595. [PubMed: 8601496]
21. White JA, Boffa MB, Jones B, Petkovich M. A zebrafish retinoic acid receptor expressed in the regenerating caudal fin. *Development.* 1994; 120:1861–1872. [PubMed: 7924993]
22. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. *Science.* 2002; 298:2188–2190. [PubMed: 12481136]
23. Raya A, Koth CM, Buscher D, Kawakami Y, Itoh T, Raya RM, et al. Activation of notch signaling pathway precedes heart regeneration in zebrafish. *Proc Natl Acad Sci U S A.* 2003; 100(Suppl 1): 11889–11895. [PubMed: 12909711]
24. Adamson PB, Gilbert EM. Reducing the risk of sudden death in heart failure with beta-blockers. *J Card Fail.* 2006; 12:734–746. [PubMed: 17174236]
25. Leone M, Magadum A, Engel FB. Cardiomyocyte proliferation in cardiac development and regeneration: a guide to methodologies and interpretations. *Am J Physiol.* 2015; 309:H1237–50.
26. Sabbah HN. Biologic rationale for the use of beta-blockers in the treatment of heart failure. *Heart Fail Rev.* 2004; 9:91–97. [PubMed: 15516856]
27. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. *Science.* 2011; 331:1078–1080. [PubMed: 21350179]
28. Hu N, Yost HJ, Clark EB. Cardiac morphology and blood pressure in the adult zebrafish. *Anat Rec.* 2001; 264:1–12. [PubMed: 11505366]
29. Ishii T, Kuwaki T, Masuda Y, Fukuda Y. Postnatal development of blood pressure and baroreflex in mice. *Auton Neurosci.* 2001; 94:34–41. [PubMed: 11775705]

30. Danneman PJ, Mandrell TD. Evaluation of five agents/methods for anesthesia of neonatal rats. *Lab Anim Sci.* 1997; 47:386–395. [PubMed: 9306312]
31. Kulandavelu S, Qu D, Sunn N, Mu J, Rennie MY, Whiteley KJ, et al. Embryonic and neonatal phenotyping of genetically engineered mice. *ILAR J.* 2006; 47:103–117. [PubMed: 16547367]
32. Christensen G, Minamisawa S, Gruber PJ, Wang Y, Chien KR. High-efficiency, long-term cardiac expression of foreign genes in living mouse embryos and neonates. *Circulation.* 2000; 101:178–184. [PubMed: 10637206]
33. Jopling C, Sleep E, Raya M, Marti M, Raya A, Belmonte JC. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature.* 2010; 464:606–609. [PubMed: 20336145]
34. Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, et al. Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. *Nature.* 2010; 464:601–605. [PubMed: 20336144]
35. Lien CL, Harrison MR, Tuan TL, Starnes VA. Heart repair and regeneration: recent insights from zebrafish studies. *Wound Repair Regen.* 2012; 20:638–646. [PubMed: 22818295]
36. Itou J, Oishi I, Kawakami H, Glass TJ, Richter J, Johnson A, et al. Migration of cardiomyocytes is essential for heart regeneration in zebrafish. *Development.* 2012; 139:4133–4142. [PubMed: 23034636]
37. Schindler YL, Garske KM, Wang J, Firulli BA, Firulli AB, Poss KD, et al. Hand2 elevates cardiomyocyte production during zebrafish heart development and regeneration. *Development.* 2014; 141:3112–3122. [PubMed: 25038045]
38. Gamba L, Harrison M, Lien CL. Cardiac regeneration in model organisms. *Curr Treat Options Cardiovasc Med.* 2014; 16:288. [PubMed: 24496965]
39. Kikuchi K. Dedifferentiation, transdifferentiation, and proliferation: mechanisms underlying cardiac muscle regeneration in zebrafish. *Curr Pathobiol Rep.* 2015; 3:81–88. [PubMed: 25722956]
40. Gemberling M, Karra R, Dickson AL, Poss KD. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. *eLife.* 2015; 4
41. Bersell K, Arab S, Haring B, Kuhn B. Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell.* 2009; 138:257–270. [PubMed: 19632177]
42. Polizzotti BD, Ganapathy B, Walsh S, Choudhury S, Ammanamanchi N, Bennett DG, et al. Neuregulin stimulation of cardiomyocyte regeneration in mice and human myocardium reveals a therapeutic window. *Sci Transl Med.* 2015; 7:281ra45.
43. D’Uva G, Aharonov A, Lauriola M, Kain D, Yahalom-Ronen Y, Carvalho S, et al. ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat Cell Biol.* 2015; 17:627–638. [PubMed: 25848746]
44. Reuter S, Soonpaa MH, Firulli AB, Chang AN, Field LJ. Recombinant neuregulin 1 does not activate cardiomyocyte DNA synthesis in normal or infarcted adult mice. *PLoS One.* 2014; 9:e115871. [PubMed: 25545368]
45. D’Uva G, Tzahor E. The key roles of ERBB2 in cardiac regeneration. *Cell Cycle.* 2015; 14:2383–2384. [PubMed: 26083142]
46. Yutzey KE. Regenerative biology: neuregulin 1 makes heart muscle. *Nature.* 2015; 520:445–446. [PubMed: 25903623]
47. Kumar A, Brockes JP. Nerve dependence in tissue, organ, and appendage regeneration. *Trends Neurosci.* 2012; 35:691–699. [PubMed: 22989534]
48. Mahmoud AI, O’Meara CC, Gemberling M, Zhao L, Bryant DM, Zheng R, et al. Nerves regulate cardiomyocyte proliferation and heart regeneration. *Dev Cell.* 2015; 34:387–399. [PubMed: 26256209]
49. White IA, Gordon J, Balkan W, Hare JM. Sympathetic reinnervation is required for mammalian cardiac regeneration. *Circ Res.* 2015
50. Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG, et al. A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell.* 2006; 127:607–619. [PubMed: 17081981]

51. Kikuchi K, Gupta V, Wang J, Holdway JE, Wills AA, Fang Y, et al. tcf21+ epicardial cells adopt non-myocardial fates during zebrafish heart development and regeneration. *Development*. 2011; 138:2895–2902. [PubMed: 21653610]
52. Kikuchi K, Holdway JE, Major RJ, Blum N, Dahn RD, Begemann G, et al. Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration. *Dev Cell*. 2011; 20:397–404. [PubMed: 21397850]
53. Kikuchi K, Poss KD. Cardiac regenerative capacity and mechanisms. *Annu Rev Cell Dev Biol*. 2012; 28:719–741. [PubMed: 23057748]
54. Chablais F, Veit J, Rainer G, Jazwinska A. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. *BMC Dev Biol*. 2011; 11:21. [PubMed: 21473762]
55. Gonzalez-Rosa JM, Martin V, Peralta M, Torres M, Mercader N. Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish. *Development*. 2011; 138:1663–1674. [PubMed: 21429987]
56. Schnabel K, Wu CC, Kurth T, Weidinger G. Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation. *PLoS One*. 2011; 6:e18503. [PubMed: 21533269]
57. Gonzalez-Rosa JM, Mercader N. Cryoinjury as a myocardial infarction model for the study of cardiac regeneration in the zebrafish. *Nat Protoc*. 2012; 7:782–788. [PubMed: 22461067]
58. Giugliano GR, Giugliano RP, Gibson CM, Kuntz RE. Meta-analysis of corticosteroid treatment in acute myocardial infarction. *Am J Cardiol*. 2003; 91:1055–1059. [PubMed: 12714146]
59. Patrono C, Baigent C. Nonsteroidal anti-inflammatory drugs and the heart. *Circulation*. 2014; 129:907–916. [PubMed: 24566065]
60. Frangogiannis NG. The mechanistic basis of infarct healing. *Antioxid Redox Signal*. 2006; 8:1907–1939. [PubMed: 17034340]
61. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. *Nat Rev Cardiol*. 2014; 11:255–265. [PubMed: 24663091]
62. Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, et al. Macrophages are required for neonatal heart regeneration. *J Clin Invest*. 2014; 124:1382–1392. [PubMed: 24569380]
63. Han C, Nie Y, Lian H, Liu R, He F, Huang H, et al. Acute inflammation stimulates a regenerative response in the neonatal mouse heart. *Cell Res*. 2015; 25:1137–1151. [PubMed: 26358185]
64. Lavine KJ, Epelman S, Uchida K, Weber KJ, Nichols CG, Schilling JD, et al. Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proc Natl Acad Sci U S A*. 2014; 111:16029–16034. [PubMed: 25349429]
65. Lien CL, Schebesta M, Makino S, Weber GJ, Keating MT. Gene expression analysis of zebrafish heart regeneration. *PLoS Biol*. 2006; 4:e260. [PubMed: 16869712]
66. Kim J, Wu Q, Zhang Y, Wiens KM, Huang Y, Rubin N, et al. PDGF signaling is required for epicardial function and blood vessel formation in regenerating zebrafish hearts. *Proc Natl Acad Sci U S A*. 2010; 107:17206–17210. [PubMed: 20858732]
67. Alcamo E, Mizgerd JP, Horwitz BH, Bronson R, Beg AA, Scott M, et al. Targeted mutation of TNF receptor I rescues the RelA-deficient mouse and reveals a critical role for NF-kappa B in leukocyte recruitment. *J Immunol*. 2001; 167:1592–1600. [PubMed: 11466381]
68. Beg AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science*. 1996; 274:782–784. [PubMed: 8864118]
69. Hayden MS, Ghosh S. Regulation of NF-kappaB by TNF family cytokines. *Semin Immunol*. 2014; 26:253–266. [PubMed: 24958609]
70. Ghosh S, Hayden MS. New regulators of NF-kappaB in inflammation. *Nat Rev Immunol*. 2008; 8:837–848. [PubMed: 18927578]
71. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science*. 1996; 274:787–789. [PubMed: 8864120]
72. Karra R, Knecht AK, Kikuchi K, Poss KD. Myocardial NF-kappaB activation is essential for zebrafish heart regeneration. *Proc Natl Acad Sci U S A*. 2015; 112:13255–13260. [PubMed: 26472034]

73. Chandrasekar B, Freeman GL. Induction of nuclear factor kappaB and activation protein 1 in postischemic myocardium. *FEBS Lett.* 1997; 401:30–34. [PubMed: 9003800]
74. Frangogiannis NG. The immune system and cardiac repair. *Pharmacol Res.* 2008; 58:88–111. [PubMed: 18620057]
75. Glaros T, Larsen M, Li L. Macrophages and fibroblasts during inflammation, tissue damage and organ injury. *Front Biosci.* 2009; 14:3988–3993.
76. Aurora AB, Olson EN. Immune modulation of stem cells and regeneration. *Cell Stem Cell.* 2014; 15:14–25. [PubMed: 24996166]
77. Coggins M, Rosenzweig A. The fire within: cardiac inflammatory signaling in health and disease. *Circ Res.* 2012; 110:116–125. [PubMed: 2223209]
78. Fang Y, Gupta V, Karra R, Holdway JE, Kikuchi K, Poss KD. Translational profiling of cardiomyocytes identifies an early Jak1/Stat3 injury response required for zebrafish heart regeneration. *Proc Natl Acad Sci U S A.* 2013; 110:13416–13421. [PubMed: 23901114]
79. Kim J, Rubin N, Huang Y, Tuan TL, Lien CL. In vitro culture of epicardial cells from adult zebrafish heart on a fibrin matrix. *Nat Protoc.* 2012; 7:247–255. [PubMed: 22262006]
80. Wang J, Karra R, Dickson AL, Poss KD. Fibronectin is deposited by injury-activated epicardial cells and is necessary for zebrafish heart regeneration. *Dev Biol.* 2013; 382:427–435. [PubMed: 23988577]
81. Chablais F, Jazwinska A. The regenerative capacity of the zebrafish heart is dependent on TGFbeta signaling. *Development.* 2012; 139:1921–1930. [PubMed: 22513374]
82. Andersen DC, Ganesalingam S, Jensen CH, Sheikh SP. Do neonatal mouse hearts regenerate following heart apex resection. *Stem Cell Rep.* 2014; 2:406–413.
83. Andersen DC, Jensen CH, Sheikh SP. Response to Sadek et al. and Kotlikoff et al. *Stem Cell Rep.* 2014; 3:3–4.
84. Jesty SA, Steffey MA, Lee FK, Breitbach M, Hesse M, Reining S, et al. c-kit+ precursors support postinfarction myogenesis in the neonatal, but not adult, heart. *Proc Natl Acad Sci U S A.* 2012; 109:13380–13385. [PubMed: 22847442]
85. Konfino T, Landa N, Ben-Mordechai T, Leor J. The type of injury dictates the mode of repair in neonatal and adult heart. *J Am Heart Assoc.* 2015; 4:e001320. [PubMed: 25628406]
86. Kotlikoff MI, Hesse M, Fleischmann BK. Comment on Do neonatal mouse hearts regenerate following heart apex resection? *Stem Cell Rep.* 2014; 3:2.
87. Sadek HA, Martin JF, Takeuchi JK, Leor J, Nie Y, Giacca M, et al. Multi-investigator letter on reproducibility of neonatal heart regeneration following apical resection. *Stem Cell Rep.* 2014; 3:1.
88. Darehzereshki A, Rubin N, Gamba L, Kim J, Fraser J, Huang Y, et al. Differential regenerative capacity of neonatal mouse hearts after cryoinjury. *Dev Biol.* 2015; 399:91–99. [PubMed: 25555840]
89. Bryant DM, O'Meara CC, Ho NN, Gannon J, Cai L, Lee RT. A systematic analysis of neonatal mouse heart regeneration after apical resection. *J Mol Cell Cardiol.* 2015; 79:315–318. [PubMed: 25533939]
90. Wang J, Panakova D, Kikuchi K, Holdway JE, Gemberling M, Burris JS, et al. The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. *Development.* 2011; 138:3421–3430. [PubMed: 21752928]
91. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, et al. Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *Proc Natl Acad Sci U S A.* 2013; 110:187–192. [PubMed: 23248315]

Table 1

Comparison of the timing of events in response to different cryoinjury severities.

Injury response	Zebrafish amputation	Zebrafish cryoinjury			Neonatal mouse cryoinjury		
		Mild	Moderate	Severe	Non-transmural	Transmural	
Endocardium activation	3hpa	3dpc ^a	–	3dpc ^a	–	–	
Epicardium activation	1–3dpa	3dpc	–	1dpc	7dpc	7dpc	
Cardiomyocyte proliferation	7dpa	3dpc	4–30dpc	3–21dpc	7dpc ^d	7dpc ^d	
Inflammatory response	Minimal (3dpa)	1–7dpc	4–7dpc	3–21dpc ^b	–	–	
Neovascularization	14dpa	–	14dpc	3–21dpc	21dpc	21dpc	
Collagen scar formation	Minimal	Minimal	7–21dpc	3–21dpc	21dpc	21dpc	
Collagen scar resolution	Yes (30dpa)	No (60dpc)	30–60dpc	90–130dpc	120dpc ^c	No	
PAI-1 expression	–	–	–	–	3dpc	3–7dpc	

The regenerative response in zebrafish follows a series of listed characterized events, from the early activation of *raldh2* expression in the endocardium to the resolution of collagen deposits. Cryoinjury shows many of the same characteristic responses, but generally incorporates a much larger inflammatory response and scar formation. Variability in the severity of the injury appears to affect the magnitude and timing of these events. Mild injury using dry ice [49] notably produces an inflammatory response, but minimal collagen scar formation. Moderate injury with a cryoprobe [47] appears to shorten the persistence collagen scarring in comparison to a more severe injury (based on tissue damage at 1dpc) [48]. However, both show formation of a relatively large collagen scar by 7dpc not seen with amputation or milder cryoinjuries. In neonatal mouse, more severe transmural injuries result in an extended period of PAI-1 expression from 3dpc beyond 7dpc and a failure to resolve the collagen scar [81].

– not examined.

^aProliferation of the endocardium.

^bPresence of apoptotic cells that might be inflammatory cells [48].

^cSignificant reduction.

^dCardiomyocyte proliferation level is not significantly different from sham operation. dpa, days post amputation; dpc, days post cryoinjury.