Published in final edited form as:

J Cardiovasc Transl Res. 2008 December 1; 1(4): 317–327. doi:10.1007/s12265-008-9065-6.

Molecular Cardiology in Translation: Gene, Cell and Chemical-Based Experimental Therapeutics for the Failing Heart

Immanuel Turner, Fikru Belema-Bedada, Joshua Martindale, DeWayne Townsend, Wang Wang, Nathan Palpant, So-chiro Yasuda, Matthew Barnabei, Ekaterina Fomicheva, and Joseph M. Metzger

Department of Integrative Biology & Physiology, University of Minnesota, Medical School, 6–125 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, USA

Abstract

Acquired and inherited diseases of the heart represent a major health care issue in this country and throughout the World. Clinical medicine has made important advancements in the past quarter century to enable several effective treatment regimes for cardiac patients. Nevertheless, it is apparent that even with the best care, current treatment strategies and therapeutics are inadequate for treating heart disease, leaving it arguably the most pressing health issue today. In this context it is important to seek new approaches to redress the functional deficits in failing myocardium. This review focuses on several recent gene, cell and chemical-based experimental therapeutics currently being developed in the laboratory for potential translation to patient care. For example, new advances in bio-sensing inducible gene expression systems offer the potential for designer cardio-protective proteins to be expressed only during hypoxia/ischemia in the heart. Stem cells continue to offer the promise of cardiac repair, and some recent advances are discussed here. In addition, discovery and applications of synthetic polymers are presented as a chemical-based strategy for acute and chronic treatment of diseased and failing cardiac tissue. Collectively, these approaches serve as the front lines in basic biomedical research, with an eye toward translation of these findings to clinically meaningful applications in cardiac disease.

Keywords

Heart; Translational; Cardiac

Greater than one million Americans die each year from myocardial infarction [1,2]. It is further disquieting to realize that even patients who survive myocardial infarction can transition to a larger and growing cohort of patients that have congestive heart failure, a progressive disease that even with optimal medical management has a 5-year survival of only 50% [1,2]. These grim statistics underscore the great need for new and efficacious strategies to address the ischemic and failing heart.

At its core, ischemic heart disease results from acute cardiac myocyte damage and the progressive remodeling of the remaining myocardium that leads to the development of heart failure. In this light, novel therapies for heart failure are being developed, from ventricular assist devices to medical management, all aimed at slowing the progression to heart failure.

While these novel therapeutic approaches are showing some benefit, there is clearly room for improvement. The recent addition of several gene-, cell-, and chemical-based therapies to the armamentarium offer new approaches to target changes in the cardiac myocyte before the remodeling and progression occur. We focus here on recent developments in gene-, cell- and chemical-based experimental strategies to enable increased mechanical performance in the ischemic and failing heart (Fig. 1).

Inducible Genetic Sensors: Development of Sentinels and Actuators

Recent gene-based experimental therapies have focused on attempting to protect the heart from ischemic injury. One of the challenges of this mode of therapy rests in the ability to precisely control transgene expression as it pertains to spatial, temporal, or physiological cues in the diseased heart. The idea of a cardiac “guardian angel” that is set into action upon appropriate signals to switch on genes and protect the heart is an attractive albeit lofty goal in the arena of inducible gene activation (Fig. 2). There are several key components that are necessary to create such a system: (1) availability of a safe and stable vector that can be administered by systemic injection and express transgenes in the heart with high efficiency and responsiveness, (2) construction of a gene switch to serve as a “sentinel” or “biosensor” that can rapidly and faithfully detect and respond to specific pathophysiological signals, and (3) the development of a physiologically relevant target gene product or pathway that when induced can enact meaningful enhancement in myocardial performance under duress [3,4]. With myocardial hypoxia providing an important substrate for the consequence of reduced blood flow to the myocardium, efforts are on-going to design and test efficacious genetic biosensors of hypoxia [3,5]. The ultimate goal of this physiologically controlled transgene expression in the heart includes silent transcription in normoxia, and rapid and robust induction in hypoxia, with the ability for expression to be turned “On” and “Off” in response to repeated bouts of hypoxia in vivo [3,4,6,7].

Induction of transgene expression in hypoxic myocardium requires development of genetic elements to sense and respond to low oxygen tension. To induce gene expression in hypoxic cells, multiple hypoxia-sensitive genetic switches have been developed, all with the general framework of the hypoxia-response element (HRE) [8,9]. Under this scaffolding, the HREs are positioned immediately upstream of a basal promoter (i.e., SV40 promoter) to drive transcription of the gene of interest. The hypoxia-response elements are *cis*-acting elements residing in the enhancers of many hypoxia-responsive endogenous genes [10]. In hypoxia a transactivating protein, hypoxia-inducible factor 1 α (HIF1 α), engages with HREs by virtue of two HIF stabilization mechanisms: (1) by the presence of an internal ribosome entry site (IRES) in the 5'UTR region of HIF1 α mRNA [11], and (2) by loss of hydroxylated prolines. HIF1 is a heterodimeric protein complex composed of HIF1- α and HIF1- β [12,13]. In cells supplied with adequate oxygen, the HIF1- α protein is rapidly degraded because of the presence of the oxygen-dependent degradation domain (ODD) in the protein [14]. The ODD contains two key proline residues at positions 402 and 564 which are modified by oxygen-sensing hydroxylases (PHD1–3) [15,16]. Once hydroxylated in normoxia, the interaction of HIF1 with von Hippel Lindau tumor suppressor targets HIF1- α for rapid degradation by the ubiquitin pathway [16, 17]. Hypoxia and several other stimuli might inhibit proline hydroxylation, thus allowing accumulation of HIF1- α in the cell [14]. Therefore, this oxygen sensitive switch is based on the combination of the interaction of the HREs and the accumulation and functionality of the HIF1 protein under hypoxic conditions.

Tang et al. [18] have described a hypoxia sensor vector encoding a chimeric transcriptional factor (GAL4-ODD-P65) composed of three components: (1) a DNA binding domain of yeast GAL4 (GAL4 DBD), (2) ODD domain of HIF1- α , and (3) activation domain of human NF- κ B p65 subunit (p65 AD). An effector vector contained six copies of the GAL4 upstream

activation sequence (GAL4 UAS) linked to the E1b TATA box driving the gene of interest [18]. The ODD domain of HIF1- α is degraded under normal oxygen conditions and stabilized in hypoxia. Thus, ideally, the GAL4-ODD-p65 only accumulates under low oxygen tension environments and subsequently transactivates the effector through the interaction between GAL4 DBD and GAL4 UAS. Finally, the effector remains silent until stimulated by the accumulation of the stabilized GAL4-ODD-p65 in hypoxia. Tang et al. [18] reported a relatively modest 11-fold difference in the elevation of the sensor in the two oxygen environments. To more specifically look at the hypoxia-inducible vector in ischemic heart disease they employed a cardiac-specific MLC-2v promoter for the sensor plasmid (pS-MLC), and an effector plasmid encoding luciferase (pE-Luc). They determined the dose response to the double vectors by examining co-transfected H9c2 cells with increasing amounts of pS while maintaining pE. In 1% vs. 20% O₂ for 24 h there was a modest three- to seven-fold induction of luciferase activity. It is uncertain whether this modest level of hypoxia induction will have efficacy for the failing heart in vivo.

Recently, Fomicheva et al. incorporated HRE concatamers together with a single proline modified ODD to form a double oxygen-sensing system (DOSVS) for gene transfer into adult cardiac myocytes and in whole heart [3] (Fig. 2). The DOSVS consisted of a sensor and effector tandem pair incorporated in viral vectors. In this system, the sensor design consisted of two or six HREs and the SV40 promoter driving the expression of the fusion protein containing the GAL4 DBD, a modified ODD domain (with only a single proline codon 564), and a p65 AD. The dual oxygen-sensing capacity of this sensor element is a result of the HREs for transcriptional regulation together with the modified ODD for posttranslational regulation [3]. The effector DOSVS combined six GAL4 binding sites upstream from a minimal promoter and a reporter gene enhanced green fluorescent protein (eGFP) or luciferase. The rationale for their study was to determine if a single proline modified ODD and concatamerized HREs signifying double oxygen-sensing function would show marked improvement with reference to currently available hypoxia-inducible systems.

To assess induction efficiency in adult cardiac myocytes, DOSVS containing 2xHRE-mp and 6xHRE-mp were used along with 6UAS-luciferase effector virus. CMV-luciferase was the control for maximum unregulated gene expression. The groups of myocytes were subjected to either 0.5% O₂ (5% CO₂, 94.5% N₂) or 20% O₂ (5% CO₂, 75% N) at 37°C for 48 h. The results showed a robust 1000-fold induction in hypoxia with little read thru detected during normoxia. The efficiency of gene transfer and expression in adult cardiac myocytes for the 6xHRE-mp sensor virus was 94±5%.

To assess fidelity of the dual oxygen-sensing system in vivo, an experimental model of myocardial infarction in the rat with chronic LAD ligation was implemented. Dual gene delivery (Sensor+Effector) was accomplished by ultrasound guided intramyocardial injection, then 24 h later, initial analysis was performed non-invasively. After initial screening, chronic LAD ligation was performed. The same initial screening was done at 24 h, with subsequent 4-h exposure to 7% O₂ for 2 and 3 days post-gene delivery. Luciferase activity increased each additional day after the episodic hypoxia challenge. The results of the bioluminescent signals were confirmed with a biochemical assay for luciferase from isolated hearts [3]. There was a marked 700-fold induction following myocardial hypoxia/ischemia, results that were comparable to CMV-luc alone. These new findings establish a highly efficacious hypoxia bio-sensing system that exceeds previous systems by ~100-fold.

This hypoxia-responsive system could have important use in the treatment of ischemic and failing hearts by up-regulating the expression of cardio-protective proteins, some of which are reviewed below.

Calcium-binding Proteins

The contractile and diastolic functions of the heart can be impaired by pathological insults that cause dysregulation of intracellular Ca^{2+} cycling. Therefore, cardiac gene transfer studies have been focused on manipulating the function of Ca^{2+} handling proteins to enhance Ca^{2+} release, to retain Ca^{2+} storage in sarcoplasmic reticulum, facilitate Ca^{2+} removal from cytoplasm, and/or lower the elevated diastolic Ca^{2+} levels. Genetic regulation of Ca^{2+} handling proteins such as increasing content of SERCA2a, sorcin, S100A1 or NCX, and those involved with removing the inhibition of PLN have been shown to mitigate diastolic and systolic dysfunction [19-22]. This approach also appears to have limitations. SERCA2a gene transfer has been shown to benefit myocyte contraction but can impair inotropic responsiveness β -adrenergic stimulation [23,24]. Overexpression of NCX can have beneficial effects in some HF models [25] but its effects may be offset by increased arrhythmogenicity [26]. Conflicting results of sorcin gene transfer in regulating contractility, relaxation and SR Ca^{2+} loading indicate that its effects may be dose-dependent [27,28].

Recently, the skeletal muscle Ca^{2+} binding protein parvalbumin (Parv) has been ectopically expressed in heart and achieves physiological significance of improved cardiac diastolic function under both normal and disease conditions. Parv is classified as a delayed Ca^{2+} buffer, which renders a unique ability to specifically bind diastolic rather than systolic Ca^{2+} under controlled-expression conditions. The low energy requirement of Parv in Ca^{2+} buffering underlies an additional advantage of Parv in redressing diastolic dysfunction of energy-deficient failing cardiac myocytes. In a number of HF animal models, Parv effectively accelerated Ca^{2+} transient decay and myocyte relaxation [29,30] and improved cardiac function in vivo [30-33]. Further, genetic engineering of distinct Parv isoforms in cardiac myocytes demonstrates the rationale for dissecting Parv's biological functions through manipulating its Ca^{2+} and Mg^{2+} buffering affinity and expression level [34]. Intriguingly, Parv gene transfer has been shown to augment energy utilization into a more efficient state, thus, improving energy function in the failing heart [31].

Cardiac Sarcomere Proteins with “Inducible” Functionality

Myocardial ischemia results from inadequate regional blood flow and leads to reduced contractility, often leading to heart failure. In the most severe case, cardiac dysfunction results in chronic ischemic cardiomyopathy and congestive heart failure. Previous studies have found that the pathophysiologic basis of cardiac contractile dysfunction associated with ischemic heart disease is, at least in part, due to calcium's reduced binding affinity for the regulatory sites of troponin C reducing the capacity for triggering the regulatory domain of troponin I [35-37]. The ternary troponin complex, troponin I, C, and T, function as the molecular on-off switch of myofilament actomyosin ATPase activity during systole and diastole and, thus, act together as a key regulator of beat to beat contractility in the heart [38]. In cases of ischemia-related myocardial pump dysfunction, the myofilaments become desensitized to calcium resulting in uncoupling of excitation-contraction (E-C) events [36,39,40]. At the sub-cellular level, E-C uncoupling is made evident by an acidosis-induced reduction in force generating capacity by the sarcomere for any given $[\text{Ca}^{2+}]_i$. Studies have shown that acidosis decreases both maximal Ca^{2+} -activated tension and the Ca^{2+} sensitivity of tension as represented by a rightward shift of the calcium dependence of force development at low pH [41-45]. Other studies have also shown that acidosis decreases myocyte maximal sarcomere length shortening amplitude and shortening velocity [42,46,47]. These experiments illustrate what is broadly understood as a desensitization of the myofilaments to activating calcium at low pH which is one of the fundamental molecular deficiencies that give rise to the cell and whole organ pathologies associated with myocardial acidosis [36,48].

In the last decade, seminal findings concerning the biochemical structure and function of troponin I have shown that the slow skeletal isoform of TnI, the early developmental cardiac isoform in mammals, is functionally resilient to low pH and consequently able to better retain proper myofibrillar contractility even in cases of severe acidosis [44,49-55]. In initial studies, mutagenesis experiments identified a critical histidine responsible for this pH-dependent functional outcome [41,42,45]. The physiological effects of this amino acid on TnI function in the heart directly reflect the unique biochemical properties of histidine's imidazole moiety which, unique among amino acids, ionizes within the physiological range ($pK_a=6.0$).

In recent work, this histidine button has been engineered into the adult cardiac isoform of TnI (cTnI A164H) providing the biophysical basis for a functional molecular rheostat of the intracellular milieu in the adult heart [42,48] (Fig. 3). A recent report of the crystal structure of cTnI places Ala164 at the critical switch domain that is key in regulating myofilament calcium activation [56]. Codon 164 is situated at the interface between the amphiphilic switch region, H3, and the C-terminal actin binding domain, H4. Together, these regions are defined as the regulatory segment. In the calcium-saturated state, the entire regulatory segment of cTnI (residues 137–210) undergoes a conformational change concomitant with electrostatic binding of the H3 domain to the conserved N-terminal hydrophobic patch of TnC. The potential implications of an ionized histidine in this position of TnI could either strengthen interactions with acidic residues on TnC or, alternatively, weaken interactions with basic residues on actin, as previously suggested [41,42]. Importantly, this engineered mutation increases myofilament sensitivity to activating calcium in a pH-dependent manner while retaining important PKA phosphorylation sites on the N-terminus of cTnI. PKA-mediated phosphorylation of TnI is a critical mediator of the β -adrenergic response in the adult heart [57]. Further studies are required to address the specific biochemistry and biophysics of cTnIA164H within the context of the thin filament. The physiological implications of this histidine modification have been studied at length [42] showing that cTnI A164H acts as a molecular rheostat maintaining systolic and diastolic pump function in models of severe acidosis and ischemic cardiomyopathy.

Targeting myofilament calcium responsiveness via manipulation of TnC and TnI interactions has been the focus of many studies seeking a mechanism for protecting cardiac function in various models of myocardial stress [42,48-61]. This is in concurrence with Mann and Bristow's view that augmentation of myofilament responsiveness to calcium would improve the force generating capacity of the sarcomere and thus redress global cardiac dysfunction [60]. In principle, cTnI A164H is ideally suited to act as a titratable inotrope showing little effect at baseline but significantly augmenting cardiac performance under conditions of cardiac stress associated with myocardial acidosis. This has reproducibly been shown to occur in various cardiomyopathic models including chronic ischemia, ischemia/reperfusion, hypoxic acidosis, and in a mouse model of age related cardiac dysfunction [42,62]. It is proposed that under mildly acidic conditions, the increasing propensity for the ionization of histidine on cTnI A164H significantly increases the sensitivity of the thin filament toward calcium allowing for continued cross bridge cycling and, importantly, maintaining cardiac performance during injury [42,48,62].

Cell-based Systems in Translation for Failing Heart

Stem cells have generated a great deal of enthusiasm among basic researchers and clinicians for their potential role in tissue repair and clinical application (Fig. 1). For example, promising experimental results have suggested the possibility of repairing damaged myocardium using adult bone-marrow-derived stem cells [63-66]. Several different populations of stem cells have been shown to reduce ventricular remodeling and increase cardiac function when injected into infarcted hearts in experimental models. However, the cellular mechanisms by which such

structural and functional recoveries are achieved are still debated [67,68]. There are at least three possibilities as to how these stem cells could be beneficial. First, transplanted stem cells may transdifferentiate into tissue specific cells. Second, they may fuse with the endogenous heart cells. Third, they may serve a paracrine function by releasing molecules such as cytokines with potential therapeutic value to neighboring cells. Overall, the progress of stem-cell therapy for heart disease has been hampered by many unaddressed biologic and technical problems that include lack of complete understanding of the ideal approach in terms of choice of cell type, time of treatment, and method of delivery. Nevertheless, therapeutic effect has been evidenced to varying degrees in models ranging from myocardial infarction to stroke [69-72].

It has been reported that adult bone-marrow-derived mesenchymal stem cells (MSCs) may differentiate into cardiac myocytes and, hence, provide a cell source suitable for treatment of the damaged myocardium [70,71]. A similar cell type, mouse bone-marrow-derived multipotent adult stem cells (mBM-MASCs), appears to be inducible to yield cells with gene expression profile akin to cardiac cells [73]. Biologically, such cells are interesting owing to their multipotency and immunomodulatory properties. A major limitation to the efficacy of cell therapy involving MSCs is the relatively poor viability of the transplanted cells resulting in reduced level of functional improvement. Genetic modification of stem or progenitor cells may represent an important strategic advancement in heart regenerative medicine. By combining gene with cell therapy, one may be able to enhance stem-cell viability and, hence, functional outcomes. Furthermore, the cells may become a vehicle for gene therapy whose secreted gene products can exert paracrine or endocrine actions that may result in further therapeutic benefits. For instance, genetically modified MSCs over-expressing the anti-apoptotic gene Akt1 (Akt-MSCs) have been shown to be more resistant to apoptosis in vitro and in vivo [74]. Furthermore, when injected into infarcted hearts, Akt-MSCs limit ventricular remodeling and improve cardiac function. Interestingly, MSCs transduced with heme oxygenase (HO)-1 survive longer when they are injected into infarcted hearts, leading to more efficient healing compared with control MSCs. Moreover HO-1 is known to have cardio-protective properties, which is due to its paracrine actions [75]. Combination of cell and gene therapy might represent a future effective strategy for myocardial repair and regeneration.

Regulation of genetically modified stem cells by hypoxia

Evidence from a variety of experimental systems has shown that hypoxia regulates the proliferation and differentiation of different stem-cell populations, including embryonic stem (ES) cells, neuronal and neural crest stem cells, hematopoietic stem cells (HSCs), and trophoblast stem cells. The direct role of hypoxia-inducible factor (HIF) in controlling these effects has been demonstrated in certain experimental settings, but as yet not tested in a majority of the stem cells types. The hypoxic responses of different stem-cell populations is consistent with the idea that oxygen levels may be an important component of particular stem-cell “niches” and that HIF activity can regulate the defining features of stem cells, including self renewal and multipotency [76]. There is evidence that circulating pools of MSCs consistently and dramatically increase in animals subjected to chronic hypoxia [77]. Therefore, developing an in vitro and in vivo model amenable to increasing MSC-mobilizing potential is another new therapeutic approach. It is worth considering a hypoxia-inducible gene therapy approach in adult stem cells for the expression of therapeutic genes to facilitate cell regeneration and survival in hypoxic regions of the heart compared to the control unmodified stem cells. The chimeric therapeutic gene is placed under the control of hypoxia-response element (HRE) based promoter and contains an oxygen-dependent degradation domain (ODD) (discussed above) and the therapeutic gene. Under hypoxic conditions, an increase in stable HIF-1 protein levels will be correlated with synthesis of the therapeutic protein. Thus, the hypoxia-modulated gene expression in mBM-MASCs has the potential to be used as a gene-based stem-cell therapy within hypoxic regions of the heart.

Genetically modified MSCs provide therapeutic potential in diseases of several vital organs through modulation of cell death (apoptosis), inflammation, fibrosis, regeneration, and angiogenesis. Experimental success has been obtained using several different modes of MSC therapy including cell transplantation, incorporation of MSCs into biocompatible devices and delivery of only the MSC-secreted molecules. However, important questions regarding the optimal and long-term delivery method of MSC therapy remain a big challenge. Transplanted MSCs have the potential benefit of homing to the site of injury and continue delivering their trophic signals. However, long-term engraftment levels appear low. Eventually, MSCs may be used as a cargo of useful molecules in addition to themselves providing trophic effects that eventually heal the damaged heart. This might provide further insight into the possible mechanism of action for therapeutic protein expression in adult stem cells for cardiovascular clinical studies and might suggest a new clinical indication that could be potentially pursued. In this light, the recent demonstration of a beating heart derived from a fully decellularized heart represents an exciting template to pursue efficacy of genetically modified stem cells for functional repair [78].

Chemical-based Experiment Therapeutics

Evidence is emerging that a new class of chemicals, classified as tri-block co-polymers, may have application in failing hearts resulting from membrane damage (Fig. 4). Duchenne muscular dystrophy (DMD) is known to result from the absence of the cytoskeletal protein dystrophin and affects both cardiac and skeletal muscles [79]. Dystrophin is a large protein that is expressed primarily in striated muscle cells. It is localized to the periphery of these cells where the N-terminal binds to the sub-membranous actin lattice and the C-terminal is associated with a complex of glycoproteins in the sarcolemmal membrane [80,81]. The bulk of the protein consists of a central rod domain consisting of 24 spectrin repeat domains. Dystrophin is hypothesized to be important in buffering the sarcolemmal membrane from the forces generated by the sarcomere. In the absence of dystrophin, the stability of the cardiac muscle membrane is compromised, such that during contractions, it is hypothesized that small holes form within the muscle membrane [82]. Given the prospect of these membrane micro tears, chemically mediated resealing holds promise as a new therapeutic approach. Tri-block co-polymers with an ABA structure are ideally suited for such a purpose. These polymers consist of a central hydrophobic domain flanked by two hydrophilic regions. Poloxamer 188 (P188) is an ABA tri-block copolymer that has been shown to reseal biological membranes of isolated cells [83-85]. Furthermore, P188 improves the mechanical stability of non-muscle cells [83,86] and facilitates resealing following mechanical puncture [85]. These membrane-protective effects of P188 suggest that it might be of therapeutic benefit in DMD, especially in the heart, where small influxes of Ca^{2+} can have significant consequences [82].

The mdx mouse, an animal model of DMD, was used to assess the acute effects of P188 on cardiac myocyte and whole heart performance. Examining the passive characteristics of membrane intact adult cardiac myocytes isolated from mdx mice revealed that mdx myocytes have an increase in passive tension during physiological extension compared to control myocytes [82]. The increased passive tension is hypothesized to result from the small influx of extracellular calcium through micro tears in the membrane. The application of P188 to these dystrophic myocytes immediately results in a complete normalization of passive tension by the preventing tears in the myocyte membrane. In addition to the increase in passive tension, mdx myocytes are very susceptible to developing terminal contracture with sarcomere length extensions beyond 2.1 μm . The addition of P188 allows mdx myocytes to tolerate physiological stretches similar to that tolerated by control myocytes.

These deficits in mdx cardiac myocyte physiology cause a significant effect within the intact organ in vivo. The hearts of mdx mice work in a volume range that is shifted towards smaller

volumes compared to control. Although stroke volume is largely maintained, systolic and more dramatically diastolic volumes are lower than that observed in control hearts. This shift to lower volumes at end diastole appears to be an organ-level adaptation to the defect in passive extension demonstrated in mdx single myocytes in vitro. This shift to lower volumes results in a relatively normal basal cardiac function; however, there is very little cardiac reserve. The absence of cardiac reserve is particularly apparent during dobutamine stress testing, during which time acute cardiac decompensation occurs in ~40% of untreated mdx mice. Systemic pretreatment with P188 normalized the changes in volumes and prevented the dobutamine induced collapse of cardiac function [82].

The correction of the dystrophic phenotype in individual cardiac myocytes by P188 results in global changes in left ventricular geometry and improvements in cardiac reserve. The therapeutic potential of P188 may extend beyond use in DMD to other cardiac diseases where dystrophin loss is a significant part of the pathophysiology. The loss of dystrophin has been implicated in the disease processes associated with X-linked cardiomyopathy, viral myocarditis, and heart failure. It will be interesting and important in future studies to ascertain whether in animal models or patients with heart failure would benefit from the membrane sealant activity of poloxamer 188.

Future Directions

The concept of gene- or protein-based systems that acclimate to the temporal, spatial, and (patho) physiological cues at the cardiac cell level has gained recent support as new strategies for the ailing myocardium (Figs. 1, 2, and 3). In principle, gene or protein functionality could be turned on/off in response to changes in the cardiac cellular milieu. Future study is needed in the tailoring of these “bio-sensing” inducible systems for heart in vivo. Target proteins in the sarcomere, and in calcium handling, hold promise as potential therapeutic targets for enhancing myocardial performance in the failing heart. It is possible that genetically modified stem cells harboring such genetic sensors would be advantageous in cardiac repair. In complement, new approaches featuring synthetic polymers could further enhance these approaches with the overall goal of translating basic biology discoveries to the bedside.

Acknowledgments

Funding by NIH, MDA, FEDS and AHA

References

1. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, et al. Heart disease and stroke statistics—2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2007;115:e69–e171. [PubMed: 17194875]
2. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, Zheng ZJ, Flegal K, O'Donnell C, Kittner S, et al. Heart disease and stroke statistics—2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2006;113:e85–e151. [PubMed: 16407573]
3. Fomicheva EV, Turner II, Edwards TG, Hoff J, Arden E, D'Alecy LG, Metzger JM. Double oxygen-sensing vector system for robust hypoxia/ischemia-regulated gene induction in cardiac muscle in vitro and in vivo. *Molecular Therapy* 2008;16(9):1594–1601. [PubMed: 18578010]
4. Tang Y, Schmitt-Ott K, Qian K, Kagiya S, Phillips MI. Vigilant vectors: adeno-associated virus with a biosensor to switch on amplified therapeutic genes in specific tissues in life-threatening diseases. *Methods* 2002;28:259–266. [PubMed: 12413425]

5. Boast K, Binley K, Iqbal S, Price T, Spearman H, Kingsman S, Kingsman A, Naylor S. Characterization of physiologically regulated vectors for the treatment of ischemic disease. *Human Gene Therapy* 1999;10:2197–2208. [PubMed: 10498251]
6. Binley K, Kan O, White J, Naylor S. Exploiting the hypoxia response. *Current Opinion In Molecular Therapeutics* 2003;5:650–656. [PubMed: 14755892]
7. Goverdhan S, Puntel M, Xiong W, Zirger JM, Barcia C, Curtin JF, Soffer EB, Mondkar S, King GD, Hu J, et al. Regulatable gene expression systems for gene therapy applications: progress and future challenges. *Molecular Therapy* 2005;12:189–211. [PubMed: 15946903]
8. Prentice H, Bishopric NH, Hicks MN, Discher DJ, Wu X, Wylie AA, Webster KA. Regulated expression of a foreign gene targeted to the ischaemic myocardium. *Cardiovascular Research* 1997;35:567–574. [PubMed: 9415303]
9. Su H, Arakawa-Hoyt J, Kan YW. Adeno-associated viral vector-mediated hypoxia response element-regulated gene expression in mouse ischemic heart model. *Proceedings of the National Academy of Sciences of the United States of America* 2002;99:9480–9485. [PubMed: 12084814]
10. Wenger RH, Stiehl DP, Camenisch G. Integration of oxygen signaling at the consensus HRE. *Sci STKE* 2005;2005:re12. [PubMed: 16234508]
11. Lang KJ, Kappel A, Goodall GJ. Hypoxia-inducible factor-1alpha mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia. *Molecular Biology of the Cell* 2002;13:1792–1801. [PubMed: 12006670]
12. Erbel PJ, Card PB, Karakuzu O, Bruick RK, Gardner KH. Structural basis for PAS domain heterodimerization in the basic helix–loop–helix-PAS transcription factor hypoxia-inducible factor. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:15504–15509. [PubMed: 14668441]
13. Wenger RH. Mammalian oxygen sensing, signalling and gene regulation. *Journal of Experimental Biology* 2000;203:1253–1263. [PubMed: 10729275]
14. Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin–proteasome pathway. *Proceedings of the National Academy of Sciences of the United States of America* 1998;95:7987–7992. [PubMed: 9653127]
15. Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* 2001;294:1337–1340. [PubMed: 11598268]
16. Krek W. VHL takes HIF's breath away. *Nature Cell Biology* 2000;2:E121–123.
17. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel–Lindau protein. *Nature Cell Biology* 2000;2:423–427.
18. Tang YL, Tang Y, Zhang YC, Agarwal A, Kasahara H, Qian K, Shen L, Phillips MI. A hypoxia-inducible vigilant vector system for activating therapeutic genes in ischemia. *Gene Therapy* 2005;12:1163–1170. [PubMed: 15800659]
19. del Monte F, Hajjar RJ. Targeting calcium cycling proteins in heart failure through gene transfer. *Journal of Physiology* 2003;546:49–61. [PubMed: 12509478]
20. Hoshijima M. Gene therapy targeted at calcium handling as an approach to the treatment of heart failure. *Pharmacology and Therapeutics* 2005;105:211–228. [PubMed: 15737405]
21. Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, Martone ME, Wang Y, Ross J Jr, Kranias EG, et al. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 1999;99:313–322. [PubMed: 10555147]
22. Most P, Pleger ST, Volkers M, Heidt B, Boerries M, Weichenhan D, Löffler E, Janssen PM, Eckhart AD, Martini J, et al. Cardiac adenoviral S100A1 gene delivery rescues failing myocardium. *Journal of Clinical Investigation* 2004;114:1550–1563. [PubMed: 15578088]
23. del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, Gwathmey JK, Rosenzweig A, Hajjar RJ. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* 1999;100:2308–2311. [PubMed: 10587333]
24. Hirsch JC, Borton AR, Albayya FP, Russell MW, Ohye RG, Metzger JM. Comparative analysis of parvalbumin and SERCA2a cardiac myocyte gene transfer in a large animal model of diastolic

- dysfunction. *American Journal of Physiology. Heart and Circulatory Physiology* 2004;286:H2314–H2321. [PubMed: 15148059]
25. Terracciano CM, Souza AI, Philipson KD, MacLeod KT. Na⁺–Ca²⁺ exchange and sarcoplasmic reticular Ca²⁺ regulation in ventricular myocytes from transgenic mice over-expressing the Na⁺–Ca²⁺ exchanger. *Journal of Physiology* 1998;512(Pt 3):651–667. [PubMed: 9769411]
 26. Pogwizd SM, Bers DM. Na/Ca exchange in heart failure: contractile dysfunction and arrhythmogenesis. *Annals of the New York Academy of Sciences* 2002;976:454–465. [PubMed: 12502595]
 27. Seidler T, Miller SL, Loughrey CM, Kania A, Burow A, Kettlewell S, Teucher N, Wagner S, Kogler H, Meyers MB, et al. Effects of adenovirus-mediated sorcin over-expression on excitation–contraction coupling in isolated rabbit cardiomyocytes. *Circulation Research* 2003;93:132–139. [PubMed: 12805242]
 28. Suarez J, Belke DD, Gloss B, Dieterle T, McDonough PM, Kim YK, Brunton LL, Dillmann WH. In vivo adenoviral transfer of sorcin reverses cardiac contractile abnormalities of diabetic cardiomyopathy. *American Journal of Physiology. Heart and Circulatory Physiology* 2004;286:H68–H75. [PubMed: 12958030]
 29. Huq F, Lebeche D, Iyer V, Liao R, Hajjar RJ. Gene transfer of parvalbumin improves diastolic dysfunction in senescent myocytes. *Circulation* 2004;109:2780–2785. [PubMed: 15173024]
 30. Michele DE, Szatkowski ML, Albayya FP, Metzger JM. Parvalbumin gene delivery improves diastolic function in the aged myocardium in vivo. *Molecular Therapy* 2004;10:399–403. [PubMed: 15294186]
 31. Sakata S, Lebeche D, Sakata N, Sakata Y, Chemaly ER, Liang LF, Tsuji T, Takewa Y, del Monte F, Peluso R, et al. Restoration of mechanical and energetic function in failing aortic-banded rat hearts by gene transfer of calcium cycling proteins. *Journal of Molecular and Cellular Cardiology* 2007;42:852–861. [PubMed: 17300800]
 32. Schmidt U, Zhu X, Lebeche D, Huq F, Guerrero JL, Hajjar RJ. In vivo gene transfer of parvalbumin improves diastolic function in aged rat hearts. *Cardiovascular Research* 2005;66:318–323. [PubMed: 15820200]
 33. Szatkowski ML, Westfall MV, Gomez CA, Wahr PA, Michele DE, DelloRusso C, Turner II, Hong KE, Albayya FP, Metzger JM. In vivo acceleration of heart relaxation performance by parvalbumin gene delivery. *Journal of Clinical Investigation* 2001;107:191–198. [PubMed: 11160135]
 34. Rodenbaugh DW, Wang W, Davis J, Edwards T, Potter JD, Metzger JM. Parvalbumin isoforms differentially accelerate cardiac myocyte relaxation kinetics in an animal model of diastolic dysfunction. *American Journal of Physiology. Heart and Circulatory Physiology* 2007;293:H1705–H1713. [PubMed: 17545482]
 35. Ding XL, Akella AB, Sonnenblick EH, Rao VG, Gulati J. Molecular basis of depression of Ca²⁺ sensitivity of tension by acid pH in cardiac muscles of the mouse and the rat. *Journal of Cardiac Failure* 1996;2:319–326. [PubMed: 8989647]
 36. Lee JA, Allen DG. Mechanisms of acute ischemic contractile failure of the heart. Role of intracellular calcium. *Journal of Clinical Investigation* 1991;88:361–367. [PubMed: 1864950]
 37. Parsons B, Szczesna D, Zhao J, Van Slooten G, Kerrick WG, Putkey JA, Potter JD. The effect of pH on the Ca²⁺ affinity of the Ca²⁺ regulatory sites of skeletal and cardiac troponin C in skinned muscle fibres. *Journal of Muscle Research and Cell Motility* 1997;18:599–609. [PubMed: 9350012]
 38. Farah CS, Reinach FC. The troponin complex and regulation of muscle contraction. *FASEB Journal* 1995;9:755–767. [PubMed: 7601340]
 39. Bers DM. Cardiac excitation–contraction coupling. *Nature* 2002;415:198–205. [PubMed: 11805843]
 40. Orchard CH, Kentish JC. Effects of changes of pH on the contractile function of cardiac muscle. *American Journal of Physiology* 1990;258:C967–C981. [PubMed: 2193525]
 41. Dargis R, Pearlstone JR, Barrette-Ng I, Edwards H, Smillie LB. Single mutation (A162H) in human cardiac troponin I corrects acid pH sensitivity of Ca²⁺-regulated actomyosin S1 ATPase. *Journal of Biological Chemistry* 2002;277:34662–34665. [PubMed: 12151382]
 42. Day SM, Westfall MV, Fomicheva EV, Hoyer K, Yasuda S, La Cross NC, D'Alecy LG, Ingwall JS, Metzger JM. Histidine button engineered into cardiac troponin I protects the ischemic and failing heart. *Nature Medicine* 2006;12:181–189.

43. Godt RE, Nosek TM. Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. *Journal of Physiology* 1989;412:155–180. [PubMed: 2600830]
44. Westfall MV, Borton AR, Albayya FP, Metzger JM. Myofilament calcium sensitivity and cardiac disease: insights from troponin I isoforms and mutants. *Circulation Research* 2002;91:525–531. [PubMed: 12242271]
45. Westfall MV, Metzger JM. Single amino acid substitutions define isoform-specific effects of troponin I on myofilament Ca^{2+} and pH sensitivity. *Journal of Molecular and Cellular Cardiology* 2007;43:107–118. [PubMed: 17602701]
46. Ricciardi L, Bottinelli R, Canepari M, Reggiani C. Effects of acidosis on maximum shortening velocity and force–velocity relation of skinned rat cardiac muscle. *Journal of Molecular and Cellular Cardiology* 1994;26:601–607. [PubMed: 8072014]
47. Ricciardi L, Bucx JJ, ter Keurs HE. Effects of acidosis on force–sarcomere length and force–velocity relations of rat cardiac muscle. *Cardiovascular Research* 1986;20:117–123. [PubMed: 3708645]
48. Day SM, Westfall MV, Metzger JM. Tuning cardiac performance in ischemic heart disease and failure by modulating myofilament function. *Journal of Molecular Medicine* 2007;85:911–921. [PubMed: 17396243]
49. Metzger JM, Michele DE, Rust EM, Borton AR, Westfall MV. Sarcomere thin filament regulatory isoforms. Evidence of a dominant effect of slow skeletal troponin I on cardiac contraction. *Journal of Biological Chemistry* 2003;278:13118–13123. [PubMed: 12551900]
50. Urboniene D, Dias FA, Pena JR, Walker LA, Solaro RJ, Wolska BM. Expression of slow skeletal troponin I in adult mouse heart helps to maintain the left ventricular systolic function during respiratory hypercapnia. *Circulation Research* 2005;97:70–77. [PubMed: 15961720]
51. Westfall MV, Rust EM, Metzger JM. Slow skeletal troponin I gene transfer, expression, and myofilament incorporation enhances adult cardiac myocyte contractile function. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94:5444–5449. [PubMed: 9144257]
52. Wolska BM, Vijayan K, Arteaga GM, Konhilas JP, Phillips RM, Kim R, Naya T, Leiden JM, Martin AF, de Tombe PP, et al. Expression of slow skeletal troponin I in adult transgenic mouse heart muscle reduces the force decline observed during acidic conditions. *Journal of Physiology* 2001;536:863–870. [PubMed: 11691878]
53. Westfall MV, Albayya FP, Turner II, Metzger JM. Chimera analysis of troponin I domains that influence Ca^{2+} -activated myofilament tension in adult cardiac myocytes. *Circulation Research* 2000;86:470–477. [PubMed: 10700453]
54. Westfall MV, Metzger JM. Troponin I isoforms and chimeras: tuning the molecular switch of cardiac contraction. *News in Physiological Sciences* 2001;16:278–281. [PubMed: 11719605]
55. Westfall MV, Metzger JM. Gene transfer of troponin I isoforms, mutants, and chimeras. *Advances in Experimental Medicine and Biology* 2003;538:169–174. [PubMed: 15098664]discussion 174
56. Takeda S, Yamashita A, Maeda K, Maeda Y. Structure of the core domain of human cardiac troponin in the Ca^{2+} -saturated form. *Nature* 2003;424:35–41. [PubMed: 12840750]
57. Metzger JM, Westfall MV. Covalent and noncovalent modification of thin filament action: the essential role of troponin in cardiac muscle regulation. *Circulation Research* 2004;94:146–158. [PubMed: 14764650]
58. Arteaga GM, Warren CM, Milutinovic S, Martin AF, Solaro RJ. Specific enhancement of sarcomeric response to Ca^{2+} protects murine myocardium against ischemia–reperfusion dysfunction. *American Journal of Physiology. Heart and Circulatory Physiology* 2005;289:H2183–H2192. [PubMed: 16024565]
59. Liou YM, Kuo SC, Hsieh SR. Differential effects of a green tea-derived polyphenol (–)-epigallocatechin-3-gallate on the acidosis-induced decrease in the Ca^{2+} sensitivity of cardiac and skeletal muscle. *Pflugers Archives* 2008;456:787–800. [PubMed: 18231806]
60. Mann DL, Bristow MR. Mechanisms and models in heart failure: the biomechanical model and beyond. *Circulation* 2005;111:2837–2849. [PubMed: 15927992]

61. Oldner A, Konrad D, Weitzberg E, Rudehill A, Rossi P, Wanecek M. Effects of levosimendan, a novel inotropic calcium-sensitizing drug, in experimental septic shock. *Critical Care Medicine* 2001;29:2185–2193. [PubMed: 11700420]
62. Palpant NJ, Day SM, Herron TJ, Converso KL, Metzger JM. Single histidine substituted cardiac troponin I confers protection from age-related systolic and diastolic dysfunction. *Cardiovascular Research*. 2008doi:10.1093/cvr/cvn198
63. Couzin J, Vogel G. Cell therapy. Renovating the heart. *Science* 2004;304:192–194. [PubMed: 15073347]
64. Mathur A, Martin JF. Stem cells and repair of the heart. *Lancet* 2004;364:183–192. [PubMed: 15246732]
65. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701–705. [PubMed: 11287958]
66. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proceedings of the National Academy of Sciences of the United States of America* 2001;98:10344–10349. [PubMed: 11504914]
67. Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: the scientific foundations of cardiac repair. *Journal of Clinical Investigation* 2005;115:572–583. [PubMed: 15765139]
68. Melo LG, Pachori AS, Kong D, Gnechi M, Wang K, Pratt RE, Dzau VJ. Molecular and cell-based therapies for protection, rescue, and repair of ischemic myocardium: reasons for cautious optimism. *Circulation* 2004;109:2386–2393. [PubMed: 15159329]
69. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001;32:1005–1011. [PubMed: 11283404]
70. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circulation Research* 2004;95:9–20. [PubMed: 15242981]
71. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002;105:93–98. [PubMed: 11772882]
72. Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, Jia ZQ. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999;100:II247–II256. [PubMed: 10567312]
73. Belema Bedada F, Technau A, Ebel H, Schulze M, Braun T. Activation of myogenic differentiation pathways in adult bone marrow-derived stem cells. *Molecular Biology of The Cell* 2005;25:9509–9519.
74. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nature Medicine* 2003;9:1195–1201.
75. Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. *Journal of The American College of Cardiology* 2005;46:1339–1350. [PubMed: 16198853]
76. Keith B, Simon MC. Hypoxia-inducible factors, stem cells, and cancer. *Cell* 2007;129:465–472. [PubMed: 17482542]
77. Rochefort GY, Delorme B, Lopez A, Herault O, Bonnet P, Charbord P, Eder V, Domenech J. Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. *Stem Cells* 2006;24:2202–2208. [PubMed: 16778152]
78. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nature Medicine* 2008;14:213–221.
79. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;51:919–928. [PubMed: 3319190]
80. Abmayr S, Gregorevic P, Allen JM, Chamberlain JS. Phenotypic improvement of dystrophic muscles by rAAV/microdystrophin vectors is augmented by Igf1 codelivery. *Molecular Therapy* 2005;12:441–450. [PubMed: 16099410]

81. Ervasti JM, Campbell KP. Membrane organization of the dystrophin–glycoprotein complex. *Cell* 1991;66:1121–1131. [PubMed: 1913804]
82. Yasuda S, Townsend D, Michele DE, Favre EG, Day SM, Metzger JM. Dystrophic heart failure blocked by membrane sealant poloxamer. *Nature* 2005;436:1025–1029. [PubMed: 16025101]
83. Clarke MS, McNeil PL. Syringe loading introduces macromolecules into living mammalian cell cytosol. *Journal of Cell Science* 1992;102(Pt 3):533–541. [PubMed: 1506433]
84. Lee RC, River LP, Pan FS, Ji L, Wollmann RL. Surfactant-induced sealing of electroporabilized skeletal muscle membranes in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 1992;89:4524–4528. [PubMed: 1584787]
85. Togo T, Alderton JM, Bi GQ, Steinhardt RA. The mechanism of facilitated cell membrane resealing. *Journal of Cell Science* 1999;112(Pt 5):719–731. [PubMed: 9973606]
86. Murhammer DW, Gooch CF. Structural features of nonionic polyglycol polymer molecules responsible for the protective effect in sparged animal cell bioreactors. *Biotechnology Progress* 1990;6:142–148. [PubMed: 1366546]

Fig. 1. Overview of experimental strategies to reverse pump dysfunction in failing heart. Use of viral mediated gene transfer, chemical-based membrane sealants, and stem cells are experimental strategies for treatment of acute and chronic heart failure. Therapies can be envisioned to be used preemptively to prevent injury induced cardiomyopathy in susceptible population. Alternatively, post-injury treatment could halt or perhaps reverse disease progression

Fig. 2.

Design and implementation of genetic biosensors in the heart. Double oxygen-sensing vector system for highly-sensitive gene activation during ischemia/hypoxia. **a** Schematic diagram of vector construction including the double oxygen sensor vector containing repeated HRE moieties and ODD element driving expression of a GAL4-ODD-P65 fusion protein and the effector vector containing repeated UAS moieties driving expression of a gene of interest. **b** Under baseline normoxic conditions GAL4 is degraded preventing expression of the effector vector. During myocardial ischemia (low oxygen tension), GAL4 is stabilized and activates effector vector gene transcription allowing for cellular accumulation of therapeutic protein protecting cardiac function. During recovery from injury oxygen tension is restored resulting

in suppression of GAL4 and inactivation of effector gene expression. *HRE* Hypoxia responsive element, *ODD* oxygen-dependent degradation domain

Fig. 3.

Protein biosensors for enhanced heart performance. Histidine-modified cardiac troponin I acts as a molecular rheostat sensing altered intracellular milieu of the injured heart. **a** The heterotrimeric troponin complex is part of the thin filament in the sarcomere and functions as the molecular switch to regulate contraction. **b** Differences in the primary amino acid sequence of troponin I led to the discovery of a critical histidine residue in ssTnI which conferred pH-dependent activation of the myofilament. This histidine was substituted for an alanine at codon 164 of cTnI (cTnI A164H) to confer this biochemical advantage to the adult cardiac TnI isoform. **c** Under baseline conditions cTnI A164H has similar functional characteristics as WT cTnI in vitro and in vivo. However, under conditions of ischemia/hypoxia stress, protonation of the histidine in cTnI A164H protects myofilament calcium responsiveness thus protecting cardiac function compared to WT cTnI

Fig. 4. Membrane sealants for failing myocytes. Membrane fragility is an important pathophysiological mechanism in heart failure. Ultimately, the failure of the membrane barrier places significant stress on an already compromised myocyte. The use of membrane sealant Poloxamer 188 (shown in figure) may have utility in limiting the consequences of membrane damage, by restoring the barrier function to the disrupted lipid bilayer