B 2296

Herz

Cardiovascular Diseases

Elektronischer Sonderdruck für

H. Rupp

Ein Service von Springer Medizin

Herz 2010 · 35:458–466 · DOI 10.1007/s00059-010-3382-7

H. Rupp · T.P. Rupp · P. Alter · N. Jung · S. Pankuweit · B. Maisch

Intraperikardiale Verfahren zur kardialen Regeneration durch Stammzellen

Notwendigkeit eines minimal-invasiven Zugangs (AttachLifter) in den normalen Herzbeutel

Organ des Bundesverbandes
Niedergelassener Kardiologen (BNK)
www.bnk.de

Organ der Arbeitsgemeinschaft
Leitende Kardiologische Krankenhausärzte e.V.
www. alkk.de

Springer Medizin

Herz 2010 · 35:458–466
DOI 10.1007/s00059-010-3382-7
Online publiziert: 14. Oktober 2010

H. Rupp[1] · T.P. Rupp[1, 2] · P. Alter[3] · N. Jung[1] · S. Pankuweit[3] · B. Maisch[3]
[1] Department of Internal Medicine – Cardiology, Experimental Cardiology Laboratory, Philipps University of Marburg, Marburg
[2] Department of Surgery, Hospital of Aschaffenburg, Aschaffenburg
[3] Department of Internal Medicine – Cardiology, UKGM GmbH, Marburg

Intrapericardial procedures for cardiac regeneration by stem cells

Need for minimal invasive access (AttachLifter) to the normal pericardial cavity

In the overloaded heart, the function of cardiomyocytes is impaired by disturbed gene expression as exemplified by inadequate SERCA2 expression [49, 51] or deficiency of highly unsaturated fatty acids [50]. As a consequence, neuroendocrine activation results in remodeling of the extracellular matrix which further impairs contractile function and coronary blood supply. Moreover, cardiomyocytes are lost by apoptosis [3] or necrosis and are replaced by collagen, i.e., replacement fibrosis. While the extent of collagen deposition can be reduced by interfering with fibrosis-promoting signals of angiotensin II or aldosterone, little progress has been made in preventing loss of cardiomyocytes or by promoting cardiac regeneration. Since adult stem cell transplantation has until now not met the ultimate goal of replacing fibrous tissue with viable myocardium in patients, the question arises whether signals required for adequate stem cell homing and differentiation are not recruited or are not present at adequate levels. In the treatment of pericardial diseases [29, 32], the pericardial sac is routinely used as a drug depot; thus, the question whether cells or agents to promote cardiac regeneration can be administered to the pericardial space is addressed. Since the normal pericardial space cannot safely be accessed with the conventional needle procedure which requires an adequate separation of pericardium from epicardium [32], novel tools that greatly reduce the risk of pericardial tamponade are described. It is proposed that these tools ("AttachLifter" combined with the principle of the "Attacher") can be used to administer extracardiac stem cells and agents for enhancing their homing. In addition, factors for activating resident cardiac stem cells in patients with small or no pericardial effusion could be administered.

Extracardiac stem cells: intrapericardial administration

Autologous adult mesenchymal stem cells from bone marrow have been used more often than muscle stem cells and data on safety have been available long before they were implanted intracardially. Bone marrow cells or blood progenitor cells are routinely given in patients after chemoradiotherapy and adverse events for the cardiovascular system have been examined. Breast cancer patients with impaired left ventricular function do not have higher complications after chemotherapy and stem cell rescue [46] and the procedure is generally considered to be safe. Case reports indicate, however, that arrhythmias and infiltration of cytotoxic T cells and cytokine release can occur [35, 42]. One could, thus, have expected that peripherally administered bone marrow stem cells migrate into injured myocardium and initiate tissue repair. One might conclude that chemotherapy interfered with cardiac stem cell transplantation or that the cardiac endpoints did not reflect the actual benefits of the administered stem cells. A more likely explanation is that the number of stem cells actually reaching the heart is low.

It was first shown by Strauer et al. [56] that selective intracoronary delivery of autologous bone marrow cells is possible and can improve left ventricular function in patients after myocardial infarction. In heart failure patients, mortality was also reduced [57]. Stem cell transplantation can also be achieved through intramyocardial (transepicardial, transendocardial) and transcoronary–venous injection. Although a greater number of stem cells reach the target area, benefits as expected from early animal experiments [11, 33, 36, 37, 38, 58] were not observed. It could be

argued that instead of acute administration to the myocardium, a more sustained delivery of stem cells in a larger number provides greater benefits.

Of particular interest in this respect is the pericardial space which in humans contains only a few milliliters of fluid with a low turnover rate. While the pericardial space is used routinely for administration of low molecular compounds in the treatment of patients with pericarditis [32] and various aspects of intrapericardial drug delivery are well examined in animals [21], it has to our knowledge not been used in a clinical trial for the administration of extracardiac stem cells. While fibroblast growth factor (mol. wt. 18 kDa) can be detected in the myocardium after intrapericardial administration, large proteins do not appear to reach the myocardium directly [16]. One might, thus, argue that there may also be barriers for stem cells. This, however, does not appear to be the case. Cell adhesion could be followed by diapedesis across the endothelial tight junctions and basement membrane allowing movement into the extracellular matrix [22].

Cardiac progenitor-like cells expanded in culture were infused into the pericardial sac of mice. The cells that trafficked into the myocardium retained their immature morphology, but were capable of undergoing injury-induced differentiation [55]. It was suggested that pericardial delivery or "pericardiomyoplasty" may also be clinically useful as a delivery strategy for cells to the heart [55]. In addition, it was shown in pigs that intrapericardially delivered autologous bone marrow cells can be detected in the myocardium [13]. In this experiment, the pericardial space was accessed via the subxiphoid approach with an epidural needle. Under fluoroscopic guidance, the needle was advanced past the parietal pericardium, whereby the position of the needle tip was confirmed by infusion of contrast medium. Bone marrow mononuclear cells stained with Hoechst 33342 were injected. In infarcted and noninfarcted pigs, bone marrow mononuclear cells were detected in the myocardium. Myocardial homing was more pro-

Abstract · Zusammenfassung

Herz 2010 · 35:458–466 DOI 10.1007/s00059-010-3382-7

H. Rupp · T.P. Rupp · P. Alter · N. Jung · S. Pankuweit · B. Maisch

Intrapericardial procedures for cardiac regeneration by stem cells. Need for minimal invasive access (AttachLifter) to the normal pericardial cavity

Abstract

In view of the only modest functional and anatomical improvements achieved by bone marrow-derived cell transplantation in patients with heart disease, the question was addressed whether the intracoronary, transcoronary–venous, and intramyocardial delivery routes are adequate. It is hypothesized that an intrapericardial delivery of stem cells or activators of resident cardiac stem cells increases therapeutic benefits. From such an intrapericardial depot, cells or modulating factors, such as thymosin β4 or Ac-SDKP, are expected to reach the myocardium with sustained kinetics. Novel tools which provide access to the pericardial space even in the absence of pericardial effusion are, therefore, described. When the pericardium becomes attached to the suction head (monitored by an increase in negative pressure), the pericardium is lifted from the epicardium ("AttachLifter"). The opening of the suction head ("Attacher") is narrowed by flexible clamps which grab the tissue and improve the vacuum seal in the case of uneven tissue. A ridge, i.e.,"needle guidance", on the suction head excludes injury to the epicardium, whereby the pericardium is punctured by a needle which resides outside the suction head. A fiberscope can be used to inspect the pericardium prior to puncture. Based on these procedures, the role of the pericardial space and the presence of pericardial effusion in cardiac regeneration can be assessed.

Keywords

Heart · Stem cells · Progenitor cells · Pericardium · AttachLifter

Intraperikardiale Verfahren zur kardialen Regeneration durch Stammzellen. Notwendigkeit eines minimal-invasiven Zugangs (AttachLifter) in den normalen Herzbeutel

Zusammenfassung

Angesichts der nur moderaten funktionellen und anatomischen Verbesserungen durch eine Stammzelltransplantation bei Herzerkrankungen stellt sich die Frage, ob die intrakoronaren, transkoronar-venösen und intramyokardialen Applikationswege ausreichen. Wir postulieren, dass eine intraperikardiale Applikation von Stammzellen bzw. Aktivatoren der residenten kardialen Stammzellen den therapeutischen Nutzen erhöht. Aus dem intraperikardialen Depot erreichen Zellen bzw. modulierende Faktoren (z. B. Thymosin β4, AC-SDKP) das Myokard in einer protrahierten Kinetik. Daher werden neue Instrumente beschrieben, die den Zugang in den Herzbeutel auch in Abwesenheit eines Perikardergusses ermöglichen. Das Perikard wird an den Ansaugkopf eines solchen Instruments (AttachLifter) angeheftet, die Anheftung wird überwacht durch einen Anstieg des Unterdrucks. Dabei wird Perikard vom Epikard angehoben („Lifter"), die Öffnung des Ansaugkopfes („Attacher") wird durch flexible Klemmen eingeengt, die das Gewebe festhalten und einen Vakuumverlust bei unebenem Gewebe verhindern. Eine geeignete Nadelführung am Ansaugkopf verhindert eine Verletzung des Epikards während der Perikardpunktion (Nadel befindet sich außerhalb des Ansaugkopfes). Zur Beurteilung der Perikardoberfläche kann vor der Punktion eine Fiberglasoptik (Perikardioskop) eingesetzt werden. Basierend auf diesen Verfahren kann die Beschaffenheit des Perikards mit oder ohne Perikarderguss bei der kardialen Regeneration bewertet werden.

Schlüsselwörter

Herz · Stammzellen · Progenitorzellen · Perikard · AttachLifter

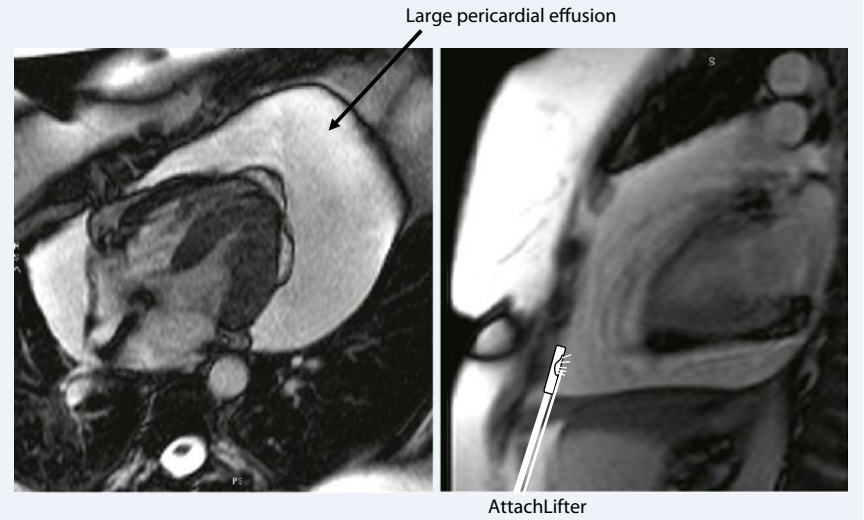

Fig. 1 ▲ Large pericardial effusion (bright area) with a typical 'swinging heart' as assessed by cardiac magnetic resonance imaging. Schematically outlined is a subxiphoid approach for pericardiocentesis with the AttachLifter

nounced in the infarction group than in animals without infarction. The largest penetration of bone marrow mononuclear cells in the myocardium occurred in the infarcted area, whereby the cells adhered to wall epithelia of small vessels. Small amounts of the cells were seen in the epicardium and myocardium in control animals. While the needle approach can apparently be used in experimental animals in the absence of pericardial effusion, it is not justified in patients when the pericardium is not adequately separated from the epicardium. Tools beyond the conventional needle are, therefore, required to minimize the risk of pericardial tamponade. In addition to administration of stem cells, the pericardial space also provides a route to modulate the activity of resident cardiac progenitor cells, whereby effects of pericardial effusion [27, 39] need to be examined.

Cardiac progenitor cells and the pericardium

Recent evidence indicates that the pericardium or the pericardial fluid provides signals for activation and gene expression of cardiac progenitor cells associated with the epicardium. This finding might not be unexpected when considering the role of the pericardium during early development of the heart. Thus, embryonic progenitor cells of the epicardium are derived from the proepicardial serosa, i.e., an extracardiac source. The proepicardial serosa is part of the pericardial wall which covers the early heart and together with the parietal pericardium contributes to the pericardial deflections. Via pericardial villi, cells (progenitor cells, vascular cells, and fibroblasts) are transferred to the heart [34]. During embryonic life, cardiomyocytes originate from epicardium and interstitial stromal cells originate from mesenchymal epicardium-derived cells. The epicardium maintains unique properties in adults. For example, epicardium-derived cells enhance cardiomyocyte proliferation, cellular alignment, and contraction, as well as the expression and cellular distribution of proteins involved in myocardial maturation [62].

When considered a part of the pericardium, the epicardium is the inner layer (visceral pericardium) of the pericardium which not only produces pericardial fluid for the movement of the inner and outer pericardial layers but has crucial influences on cardiac progenitor cells [23]. It was shown that the pericardial fluid mediates reactivation of the embryonic program in epicardial cells after myocardial infarction. These cells expressed c-kit, an antigen which identifies a stem cell population within the adult myocardium capable of differentiating toward the vascular and myocardial lineages.

It was also observed that an intact pericardial sac was associated with a thicker infarcted LV wall containing foci of regeneration and partially prevented left ventricular function deterioration [24]. Since the murine pericardium comprises only a thin mesothelial cell layer, mechanical influences of the parietal pericardium are unlikely. It was concluded that other factors, e.g., pericardial fluid accumulation, mediate epicardial c-kit+ cell activation [24]. In gene expression studies of epicardial cells from infarcted hearts with or without an intact pericardial cavity, the epicardial expression of genes involved in tissue repair and the embryonic epicardial-related genes *Tbx18* (T-box transcription factor 18), *WT1* (Wilms Tumor 1), and *RALDH2* (retinaldehyde dehydrogenase 2) were enhanced when the pericardial sac was intact.

Why is cardiac regeneration not effective as expected?

The extracellular defect hypothesis

One might hypothesize that the currently administered extracardiac adult or fetal stem cells [10] have an impaired potential of restoring the complex architecture of the myocardium. It might, however, also be that pathways, which are needed for the physiological regeneration or the continued replacement of senescent cells [17], are disturbed by fibrosis or ischemia. This physiological regeneration appears to involve a small pool of resident cardiac stem cells [59, 63]. The replicating cardiac stem cells give rise to one daughter and one daughter committed cell. By this mechanism, the pool of primitive cardiac stem cells is preserved, and a cardiomyocyte progeny is generated together with endothelial and smooth muscle cells [59]. The cells were found in specific sites under the epicardium and close to the origin of the coronary arteries and aorta [43]. They were grouped in niches which also involve supporting cells, such as mature cardiomyocytes and fibroblasts, and connections exist to the extracellular matrix.

It has been pointed out that in injuries affecting large areas as in myocardi-

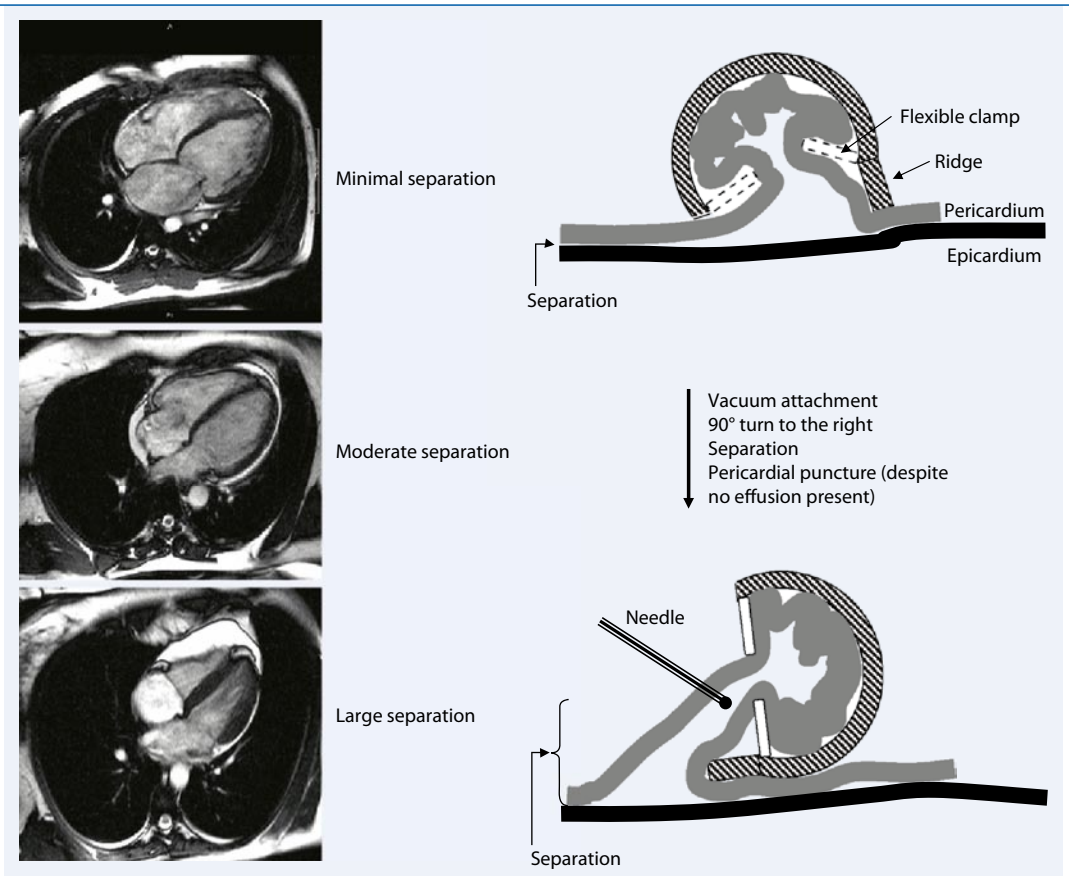

Fig. 2 ▶ Pericardial cavities with minimal (*top left*), moderate (*middle left*), or large (*bottom left*) separation of the pericardium from the epicardium. The separation by effusion is mimicked in the AttachLifter procedure by lifting the pericardium from the epicardium after pericardial attachment. A ridge serves as needle guidance which excludes injury to the ventricular wall during pericardial puncture

al infarction, the network of interstitial Cajal-like cells (ICLCs) is broken [43]. It was suggested that the ICLCs network is the scaffold capable of guiding differentiating stem cells irrespective of their origin. ICLCs could also secrete extracellular matrix molecules functioning as signaling routes. Cardiomyocytes with immature features show a variable degree of immaturity, resembling mesenchymal cells, ICLCs, or mature cardiomyocytes. Immature cardiomyocytes also exhibited spatial relationships with ICLCs.

Hypothesis of an imbalance between the inhibition and activation signals

The inadequate mobilization of resident progenitor cells for their differentiation into cardiomyocytes and vascular smooth muscle cells could be overcome not only by recruiting activation signals but also by interfering with inhibition signals. For hematopoiesis, a number of inhibitory molecules have been identified, including cytokines and peptides, such as Ac-SDKP (seraspenide) [14]. Ac-SDKP inhibits the proliferation of hematopoietic stem cells and committed progenitors [19]. Ac-SDKP, a tetrapeptide N-acetyl-Ser-Asp-Lys-Pro, is degraded by angiotensin I-converting enzyme (ACE) and is increased after ACE inhibition [44]. As outlined previously [48], ACE inhibition might also modulate cardiac stem cell regeneration by increasing the level of Ac-SDKP. In addition to putative effects on stem cells, Ac-SDKP affects angiotensin pathways. Ac-SDKP has an inhibitory action on ACE, thereby, reducing the formation of angiotensin II [12]. The contractile response of the rat aorta to angiotensin I was reduced by Ac-SDKP arising from a lower angiotensin II formation [12]. In accordance with this observation would be findings that Ac-SDKP exhibits antifibrotic effects. In rats with renal hypertension, Ac-SDKP did not interfere with left ventricular hypertrophy but blunted the increase in proliferating cell nuclear antigen- and monocyte/macrophage-positive cells [45]; the increase in interstitial collagen fraction was also prevented by Ac-SDKP. In renin-independent hypertension (aldosterone salt), Ac-SDKP markedly prevented cardiac and renal fibrosis [41]. Ac-SDKP also has direct effects on fibroblasts. Ac-SDKP suppressed differentiation of cardiac fibroblasts into myofibroblasts, probably by inhibiting the TGF-beta/Smad/ERK1/2 signaling pathway [40].

Ac-SDKP is derived from the 43 amino acid peptide thymosin β4 (Tβ4) which has cardioprotective properties in acute myocardial infarction [47]. It was proposed that some of the therapeutically beneficial effects of Tβ4 may be due to action of Ac-SDKP [47]. Since intrapericardially administered fibroblast growth factor of mol. wt. 18 kDa was detected in myocardium, the extremely water-soluble polypeptide Tβ4 (mol. wt. 5 kDa) and Ac-SDKP (mol. wt. 487 Da) are also expected to reach the myocardium directly. The further examination of Ac-SDKP with respect to modulatory influences of cardiac stem cells is also of great interest since Tβ4 is not only involved in angiogenesis in the developing embryo but also in the adult heart [54].

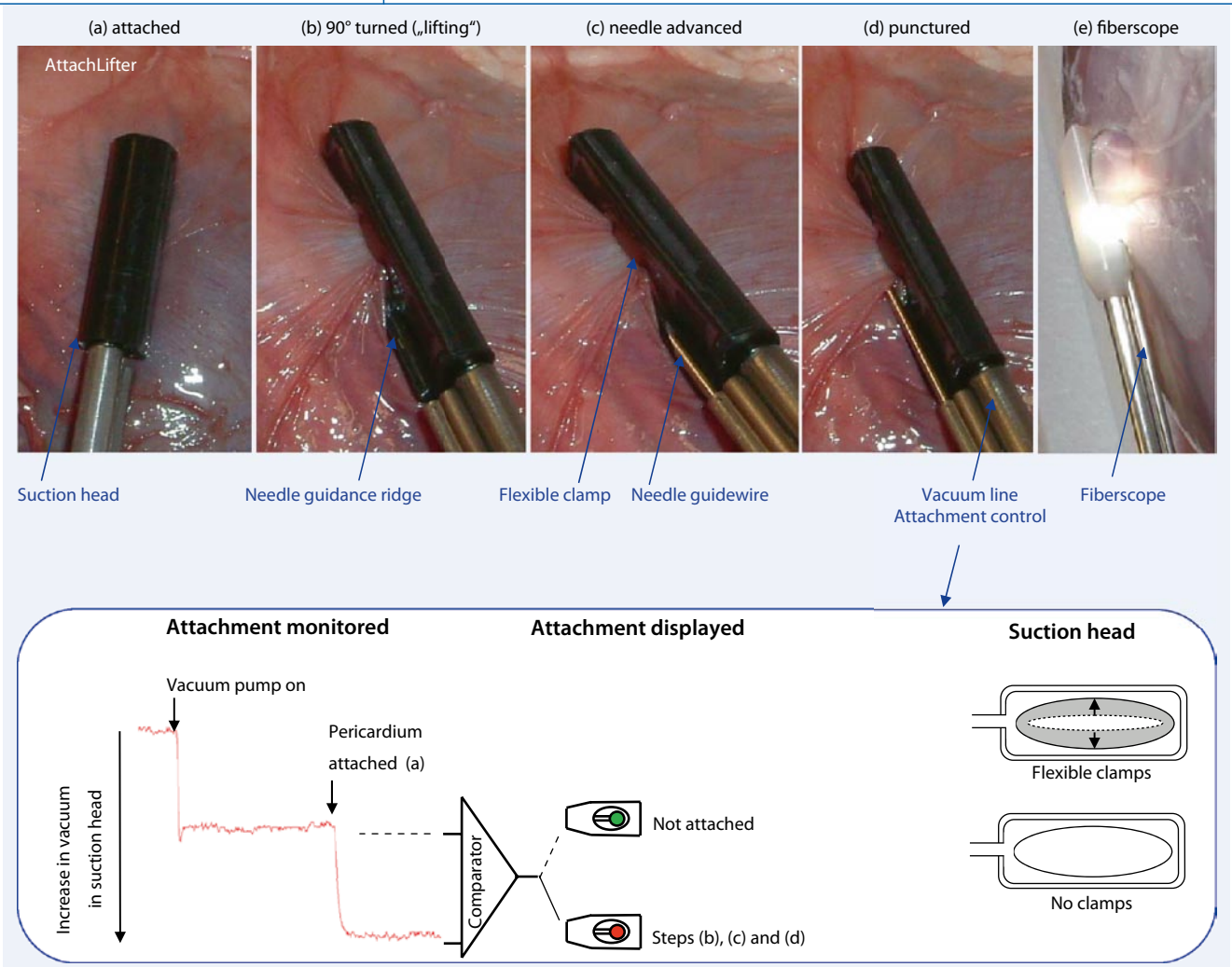

Fig. 3 ▲ Steps involved in pericardial puncture by the AttachLifter and monitoring of pericardial attachment. In the tool on the upper right (**e**), a fiberscope (11284A Vascular-Fiberscope, Karl-Storz Endoscopy Inc., Tuttlingen, Germany) was used to inspect the tissue before puncturing the pericardium. Tissue attachment is monitored by an increase in negative pressure (vacuum) which is displayed by optical and acoustic means. Tissue attachment is greatly improved by flexible clamps covering the opening of the suction head

Thus, Tβ4 stimulated outgrowth of epicardial cells which display an epithelial morphology and are positive for epicardial-specific transcription factor, epicardin/TCF21, and for proteins associated with embryonic epicardium, such as WT-1, TBX18, and GATA-5 (binds to the DNA sequence "GATA"). Using Tβ4 to stimulate quiescent adult epicardium-derived progenitor cells, up to 3000 cells were observed as outgrowth from heart pieces [53]. In our ongoing work, the influence of intrapericardially administered stem cells/Ac-SDKP in rats with or without pericardial inflammation is being examined.

New tools for intrapericardial administration of stem cells or activators

For delivering extracardiac stem cells and/or activators of resident cardiac stem cells, we addressed the question whether minimally invasive access to the pericardial space can be achieved. It is timely to invest and strengthen this line of minimally invasive access to the pericardial sac for several reasons: extensive experience has been accumulated with respect to conventional access to the pericardial sac using a Tuohy needle. The pericardial sac provides the unique opportunity of delivering a drug bolus, thereby, bypassing unwanted systemic effects [26, 28, 31, 52]. This requires, however, pericardial effusion which separates the pericardium from the epicardium and, thus, prevents puncture of the myocardium leading to cardiac tamponade. The needle technique is, therefore, not applicable to patients with cardiac overload but normal pericardium. Extensive experience has also been gathered during testing of the PerDUCER tool [25], which differs in crucial aspects from the newly developed AttachLifter.

The PerDUCER involves manual suction with a syringe for attaching the pericardium to the suction head prior to puncture with a needle. In a study performed with the PerDUCER, success-

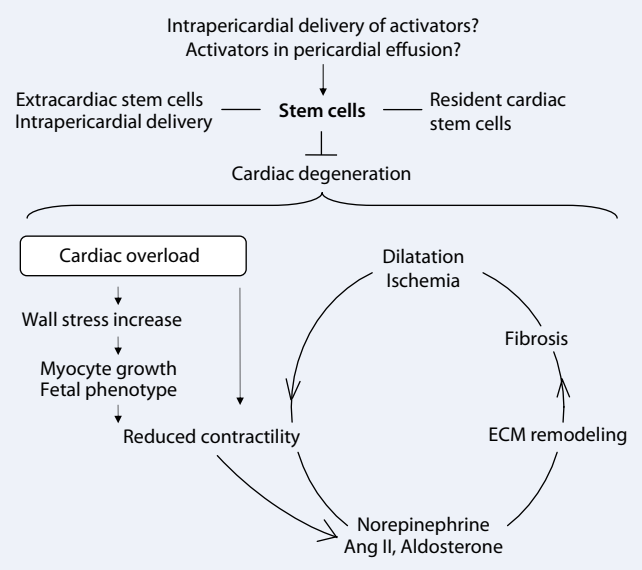

Fig. 4 ▲ Schematic of a vicious cycle typically operating during progression of heart failure. Since current therapy can often not prevent cardiac degeneration, procedures which can induce regeneration involving extracardiac stem cells or resident cardiac stem cells are required. Emphasis is placed on the intrapericardial administration of stem cells and/or activators of resident cardiac stem cells. *Ang II* angiotensin II

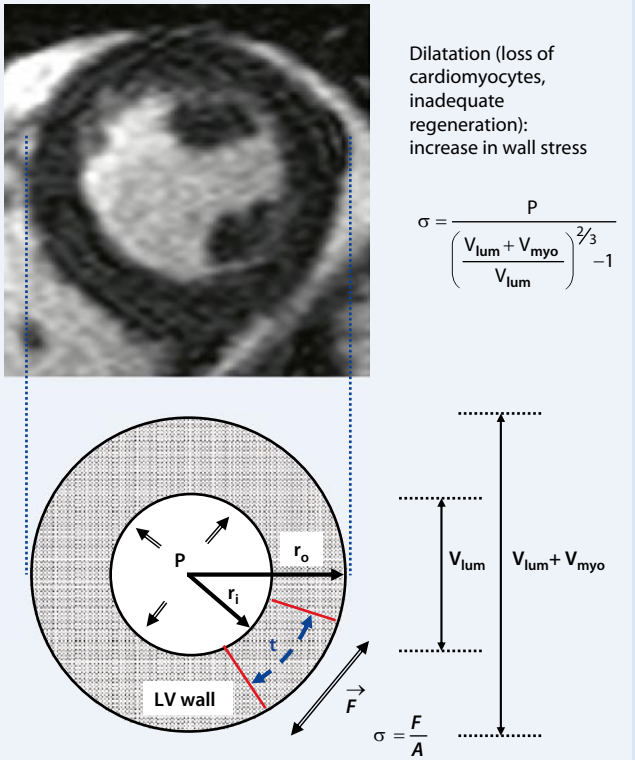

Fig. 5 ▲ Short-axis cardiac view obtained by cardiac magnetic resonance imaging (*top*). Thick-walled sphere model of the left ventricle used for calculating LV wall stress based on myocardial wall volume (V_{myo}), cavity volume (V_{lum}), and pressure (P) (*bottom*). Intraventricular pressure leads to radial and tangential (t) distending forces (F) on the myocardium which means strain of an arbitrary volume element (symbolized between *red* lines). Influenced by active and passive mechanical properties, wall stress (σ) is defined as ratio of orthogonal forces acting on a cross-section area (A) of the ventricular wall. r_i inner radius, r_o outer radius. For details, see Alter et al. [6]

ful access to the pericardial space was achieved in 2 out of 6 patients [30, 31]. A reason for the failure could be that the attachment to the pericardium was achieved only by manual suction with a syringe and that parallel to the suction, the needle had to be advanced for puncturing the attached pericardium. Any loss of attachment is expected to result in failure of pericardial puncture. Also thickened pericardium would fill out the suction head and the needle would, thus, not enter the pericardial space.

We developed, therefore, the AttachLifter for accessing the pericardial space irrespective of the pericardial thickness and presence of pericardial effusion. The tool is used in the standard subxiphoid approach and the suction head becomes attached to the pericardium by vacuum in the suction head (**Fig. 1**). The rationale of the AttachLifter is to mimic by mechanical means the separation of pericardium from epicardium seen in patients with pericardial effusion (**Fig. 2**). Successful pericardial attachment results in an increased negative pressure which is monitored and displayed by various means (thus, the term "Attacher"). A pressure recording showing the increase in negative pressure upon attachment is given in **Fig. 3**. Contrary to the PerDUCER, the pericardium is punctured outside the suction head, whereby the pericardium is lifted away from the epicardium (therefore, the term AttachLifter). In this approach (**Fig. 3**), the suction head has flexible clamps which grab the tissue more effectively and also provide a seal when a negative pressure is applied. The suction head is turned by approx. 90° to the right and the needle which is outside the vacuum channel, is pushed forward into the "tent", thereby entering the pericardial space. A so-called "safety ridge" or needle guidance is present which excludes injury to the epicardium. In case of markedly thickened tissue, the forwarded needle is left in the tissue and the suction head is turned further to the right and a stiff guidewire is pushed through a guide tube (at a 90° angle relative to the longitudinal suction head) through the firmly attached pericardium into the pericardial space (not shown). The tools for pericardial access and navigation have been described in detail in the patent applications WO/2005/016157, WO/2007/062632, and WO/2008/071367. The approach is not only promising in the context of stem cell implantation but also in the admin-

istration of nitric oxide donors, which in swine reduce luminal narrowing after coronary angioplasty [9], or the intrapericardial infusion of *n*-3 highly unsaturated fatty acids for reducing malignant arrhythmias and infarct size [64].

Cardiac regeneration by stem cells—focus on dilatation

Although various regenerative mechanisms of stem cells in heart failure have been implied [18], beneficial effects on adverse chamber dilatation are still not well understood. Thus, a meta-analysis including 999 patients in 18 trials showed an improvement of cardiac function and regression of left ventricular (LV) dilatation following stem cell therapy [1]. In addition to an increase in LV ejection fraction, a decrease in LV end-systolic volume and a trend for a decrease in the LV end-diastolic volume were found. Thus, cardiac regeneration by stem cells occurs at various levels of extracellular and cellular organization of the heart and is also expected to interfere with the vicious cycle involving neuroendocrine activation (**Fig. 4**).

Beyond regression of LV dilatation per se, these effects would be crucial for reduction of LV wall stress, which is supposed to be of major prognostic importance in dilative heart failure. LV wall stress is predominantly determined by LV volume, myocardial mass, and intraventricular pressure [4, 6]. A reduction of LV volume leads to a decrease of wall stress. Since wall stress cannot be measured directly, appropriate models have been developed for its approximation. Routinely, a thick-walled sphere model based on LV measurements as derived from cardiac magnetic resonance imaging can be used for obtaining LV wall stress in patients ([6, 8], **Fig. 5**). Increased wall stress is associated with a release of B-type natriuretic peptide and can, thus, also be used as a diagnostic criterion in heart failure [7, 8]. Furthermore, a rise in wall stress is associated with an altered autonomic tone as reflected by reduced heart rate variability [2, 5]. Any effects of stem cells on dilatation should be seen also in the context of arrhythmogenesis. Thus, increased myocardial stretch and neurohumoral changes associated with ventricular dilatation predispose to ventricular arrhythmias, e.g., mediated by stretch-activated ion channels [15, 20, 60, 61].

Corresponding address

Prof. H. Rupp
Department of Internal Medicine – Cardiology,
Experimental Cardiology Laboratory,
Philipps University of Marburg
Karl-von-Frisch-Str. 1, 35032 Marburg
Deutschland
rupp@staff.uni-marburg.de

Acknowledgments. The study was supported by the Marburg Cardiac Promotion Society.

Conflict of interest. The corresponding author states that there are no conflicts of interest.

References

1. Abdel-Latif A, Bolli R, Tleyjeh IM et al (2007) Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 167:989–997
2. Alter P, Grimm W, Vollrath A et al (2006) Heart rate variability in patients with cardiac hypertrophy–relation to left ventricular mass and etiology. Am Heart J 151:829–836
3. Alter P, Rupp H, Maisch B (2006) Activated nuclear transcription factor kappaB in patients with myocarditis and dilated cardiomyopathy–relation to inflammation and cardiac function. Biochem Biophys Res Commun 339:180–187
4. Alter P, Rupp H, Maisch B (2008) Assessment and relevance of ventricular wall stress in heart failure. Eur Heart J 29:2316
5. Alter P, Rupp H, Rominger MB et al (2009) Depression of heart rate variability in patients with increased ventricular wall stress. Pacing Clin Electrophysiol 32(Suppl 1):S26–S31
6. Alter P, Rupp H, Rominger MB et al (2008) A new methodological approach to assess cardiac work by pressure-volume and stress-length relations in patients with aortic valve stenosis and dilated cardiomyopathy. Pflugers Arch 455:627–636
7. Alter P, Rupp H, Rominger MB et al (2008) B-type natriuretic peptide and wall stress in dilated human heart. Mol Cell Biochem 314:179–191
8. Alter P, Rupp H, Rominger MB et al (2007) Relation of B-type natriuretic peptide to left ventricular wall stress as assessed by cardiac magnetic resonance imaging in patients with dilated cardiomyopathy. Can J Physiol Pharmacol 85:790–799
9. Baek SH, Hrabie JA, Keefer LK et al (2002) Augmentation of intrapericardial nitric oxide level by a prolonged-release nitric oxide donor reduces luminal narrowing after porcine coronary angioplasty. Circulation 105:2779–2784
10. Benetti F, Penherrera E, Maldonado T et al (2010) Direct myocardial implantation of human fetal stem cells in heart failure patients: long-term results. Heart Surg Forum 13:E31–E35
11. Bittner RE, Schofer C, Weipoltshammer K et al (1999) Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl) 199:391–396
12. Boulanger CM, Ezan E, Masse F et al (1998) The hemoregulatory peptide N-acetyl-ser-asp-lys-pro impairs angiotensin I-induced contractions in rat aorta. Eur J Pharmacol 363:153–156
13. Branco E, Fioretto ET, Cabral R et al (2009) Myocardial homing after intrapericardial infusion of bone marrow mononuclear cells. Arq Bras Cardiol 93:e50–e53
14. Carde P (1994) Inhibitors of hematopoiesis: from physiology to therapy. Bull Acad Natl Med 178:793–803
15. Franz MR, Cima R, Wang D et al (1992) Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. Circulation 86:968–978
16. Hermans JJ, Essen H van, Struijker-Boudier HA et al (2002) Pharmacokinetic advantage of intrapericardially applied substances in the rat. J Pharmacol Exp Ther 301:672–678
17. Hosoda T, Kajstura J, Leri A, Anversa P (2010) Mechanisms of myocardial regeneration. Circ J 74:13–17
18. Ichim TE, Solano F, Lara F et al (2010) Combination stem cell therapy for heart failure. Int Arch Med 3:5
19. Jackson JD, Yan Y, Ewel C, Talmadge JE (1996) Activity of acetyl-n-ser-asp-lys-pro (AcSDKP) on hematopoietic progenitor cells in short-term and long-term murine bone marrow cultures. Exp Hematol 24:475–481
20. Kelly D, Mackenzie L, Hunter P et al (2006) Gene expression of stretch-activated channels and mechanoelectric feedback in the heart. Clin Exp Pharmacol Physiol 33:642–648
21. Kolettis TM, Kazakos N, Katsouras CS et al (2005) Intrapericardial drug delivery: pharmacologic properties and long-term safety in swine. Int J Cardiol 99:415–421
22. Kollar K, Cook MM, Atkinson K, Brooke G (2009) Molecular mechanisms involved in mesenchymal stem cell migration to the site of acute myocardial infarction. Int J Cell Biol 2009:904682
23. Limana F, Bertolami C, Mangoni A et al (2010) Myocardial infarction induces embryonic reprogramming of epicardial c-kit(+) cells: role of the pericardial fluid. J Mol Cell Cardiol 48:609–618
24. Limana F, Zacheo A, Mocini D et al (2007) Identification of myocardial and vascular precursor cells in human and mouse epicardium. Circ Res 101:1255–1265
25. Macris MP, Igo SR (1999) Minimally invasive access of the normal pericardium: initial clinical experience with a novel device. Clin Cardiol 22:I36–I39
26. Maisch B, Drude L (1992) Pericardioscopy—a new window to the heart in inflammatory heart diseases. Herz 17:71–78
27. Maisch B, Ristic A, Pankuweit S (2002) Intrapericardial treatment of autoreactive pericardial effusion with triamcinolone. The way to avoid side effects of systemic corticosteroid therapy. Eur Heart J 23:1503–1508

28. Maisch B, Ristic A, Pankuweit S et al (2002) Neoplastic pericardial effusion. Efficacy and safety of intrapericardial treatment with cisplatin. Eur Heart J 23:1625–1631
29. Maisch B, Ristic A, Seferovic PM, Tsang T (2010) Interventional pericardiology. Springer, Berlin
30. Maisch B, Ristic AD, Rupp H, Spodick DH (2001) Pericardial access using the PerDUCER and flexible percutaneous pericardioscopy. Am J Cardiol 88:1323–1326
31. Maisch B, Ristic AD, Seferovic PM, Spodick DH (2000) Intrapericardial treatment of autoreactive myocarditis with triamcinolon. Successful administration in patients with minimal pericardial effusion. Herz 25:781–786
32. Maisch B, Seferovic PM, Ristic AD et al (2004) Guidelines on the diagnosis and management of pericardial diseases executive summary; the task force on the diagnosis and management of pericardial diseases of the European society of cardiology. Eur Heart J 25:587–610
33. Malouf NN, Coleman WB, Grisham JW et al (2001) Adult-derived stem cells from the liver become myocytes in the heart in vivo. Am J Pathol 158:1929–1935
34. Manner J (1992) The development of pericardial villi in the chick embryo. Anat Embryol (Berl) 186:379–385
35. Nagashima H, Kawashiro-Hirata N, Imamura K et al (2000) Congestive heart failure after peripheral blood stem cell transplantation: role of cytokines. Jpn Circ J 64:382–384
36. Orlic D, Kajstura J, Chimenti S et al (2001) Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann N Y Acad Sci 938:221–229; discussion 229–230
37. Orlic D, Kajstura J, Chimenti S et al (2001) Bone marrow cells regenerate infarcted myocardium. Nature 410:701–705
38. Orlic D, Kajstura J, Chimenti S et al (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci U S A 98:10344–10349
39. Pankuweit S, Wadlich A, Meyer E et al (2000) Cytokine activation in pericardial fluids in different forms of pericarditis. Herz 25:748–754
40. Peng H, Carretero OA, Peterson EL, Rhaleb NE (2010) Ac-SDKP inhibits transforming growth factor-beta1-induced differentiation of human cardiac fibroblasts into myofibroblasts. Am J Physiol Heart Circ Physiol 298:H1357–H1364
41. Peng H, Carretero OA, Raij L et al (2001) Antifibrotic effects of N-acetyl-seryl-aspartyl-lysyl-proline on the heart and kidney in aldosterone-salt hypertensive rats. Hypertension 37:794–800
42. Platzbecker U, Klingel K, Thiede C et al (2001) Acute heart failure after allogeneic blood stem cell transplantation due to massive myocardial infiltration by cytotoxic T cells of donor origin. Bone Marrow Transplant 27:107–109
43. Popescu LM, Gherghiceanu M, Manole CG, Faussone-Pellegrini MS (2009) Cardiac renewing: interstitial Cajal-like cells nurse cardiomyocyte progenitors in epicardial stem cell niches. J Cell Mol Med 13:866–886
44. Rasoul S, Carretero OA, Peng H et al (2004) Antifibrotic effect of Ac-SDKP and angiotensin-converting enzyme inhibition in hypertension. J Hypertens 22:593–603
45. Rhaleb NE, Peng H, Yang XP et al (2001) Long-term effect of N-acetyl-seryl-aspartyl-lysyl-proline on left ventricular collagen deposition in rats with 2-kidney, 1-clip hypertension. Circulation 103:3136–3141
46. Rose M, Lee FA, Gollerkeri A et al (2000) The feasibility of high-dose chemotherapy in breast cancer patients with impaired left ventricular function. Bone Marrow Transplant 26:133–139
47. Rossdeutsch A, Smart N, Riley PR (2008) Thymosin beta4 and Ac-SDKP: tools to mend a broken heart. J Mol Med 86:29–35
48. Rupp H, Alter P, Maisch B (2002) Stem cells for the failing heart—does ACE inhibition interfere? J Clin Res 8:10–13
49. Rupp H, Rupp TP, Alter P, Maisch B (2006) Acute heart failure–basic pathomechanism and new drug targets. Herz 31:727–735
50. Rupp H, Rupp TP, Alter P, Maisch B (2010) Inverse shift in serum polyunsaturated and monounsaturated fatty acids is associated with adverse dilatation of the heart. Heart 96:595–598
51. Rupp H, Rupp TP, Maisch B (2005) Fatty acid oxidation inhibition with PPARalpha activation (FOXIB/PPARalpha) for normalizing gene expression in heart failure? Cardiovasc Res 66:423–426
52. Seferovic PM, Ristic AD, Maksimovic R et al (2000) Flexible percutaneous pericardioscopy: inherent drawbacks and recent advances. Herz 25:741–747
53. Smart N, Riley PR (2009) Derivation of epicardium-derived progenitor cells (EPDCs) from adult epicardium. Curr Protoc Stem Cell Biol Chapter 2:Unit2C
54. Smart N, Risebro CA, Melville AA et al (2007) Thymosin beta-4 is essential for coronary vessel development and promotes neovascularization via adult epicardium. Ann N Y Acad Sci 1112:171–188
55. Steele A, Jones OY, Gok F et al (2005) Stem-like cells traffic from heart ex vivo, expand in vitro, and can be transplanted in vivo. J Heart Lung Transplant 24:1930–1939
56. Strauer BE, Brehm M, Zeus T et al (2001) Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction. Dtsch Med Wochenschr 126:932–938
57. Strauer BE, Yousef M, Schannwell CM (2010) The acute and long-term effects of intracoronary stem cell transplantation in 191 patients with chronic heart failure: the STAR-heart study. Eur J Heart Fail 12:721–729
58. Tomita S, Li RK, Weisel RD et al (1999) Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 100:II247–II256
59. Urbanek K, Cesselli D, Rota M et al (2006) Stem cell niches in the adult mouse heart. Proc Natl Acad Sci U S A 103:9226–9231
60. Wang Z, Taylor LK, Denney WD, Hansen DE (1994) Initiation of ventricular extrasystoles by myocardial stretch in chronically dilated and failing canine left ventricle. Circulation 90:2022–2031
61. Watanabe H, Murakami M, Ohba T et al (2008) TRP channel and cardiovascular disease. Pharmacol Ther 118:337–351
62. Weeke-Klimp A, Bax NA, Bellu AR et al (2010) Epicardium-derived cells enhance proliferation, cellular maturation and alignment of cardiomyocytes. J Mol Cell Cardiol 49:606–616
63. Winter EM, Gittenberger-de Groot AC (2007) Epicardium-derived cells in cardiogenesis and cardiac regeneration. Cell Mol Life Sci 64:692–703
64. Xiao YF, Sigg DC, Ujhelyi MR et al (2008) Pericardial delivery of omega-3 fatty acid: a novel approach to reducing myocardial infarct sizes and arrhythmias. Am J Physiol Heart Circ Physiol 294:H2212–H2218