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REVIEW

Pericardial fluid: an underrated molecular library of heart conditions and a potential vehicle for cardiac therapy

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Abstract
The remote but heart-encircling location of pericardial fluid confers this biofluid unique properties. Once past the limitation of the invasive collection, for instance, on occasion of heart surgery or pericardiocentesis, the scrutiny of pericardial fluid content can be of great interest in cardiovascular research. This liquid concentrates many heart-derived factors, thus enclosing several surrogate markers for the diagnosis or prognosis of a large spectrum of diseases either pericardial (e.g. malignant or tuberculous pericarditis) or non-pericardial/heart diseases (e.g. coronary artery disease or valvular heart diseases). Herein, for the first time, the molecular knowledge of pericardial fluid is reviewed, through an in-depth literature search and mining, and then translated into a network map of the diseases influencing pericardial fluid composition. The suitability of pericardial fluid for biomarker research could be demonstrated by evident molecular profiles between different conditions as well as by stronger correlations to cardiac structural and functional parameters, fainter or lacking in plasma/serum. Also highlighted here are the results of mechanistic research conducted with pericardial fluid in several hot topics of research, such as chronotropy, inotropy, coronary perfusion and cardiac electrophysiology. Moreover, the progress in intrapericardial therapeutics, motivated by pericardial fluid's low clearance rates, higher efficiency and lesser risk of systemic effects over conventional delivery methods, is surveyed and discussed.

Keywords Heart disease · Pericardial fluid · Systems medicine · Biomarker · Intrapericardial therapeutics

Introduction

Cardiology has witnessed tremendous progress in the last decades. Current knowledge of the pathophysiological mechanisms underlying cardiac disease and heart's adaptation to pharmacotherapy and surgical intervention is growing but far from complete. Particularly unexplored is the role of pericardial fluid on cardiac pathology and its diagnostic, prognostic and therapeutic value. Despite its existence being recognised early by Hippocrates in Ancient Greece, who described pericardial fluid as "*resembling urine*" [144], not long ago (more precisely in the end of the twentieth century) researchers have paid attention to the potential diagnostic value of this biofluid for heart diseases and not only those directly affecting the pericardium with evident clinical manifestations. Advances in the molecular biology and omics sciences are expected to deliver unprecedented knowledge of the molecular features of this biofluid. However, large-scale screening of pericardial fluid proteome [178] and miRNome [85] just started. Moreover, the acknowledgment of the heart not solely as a muscle but also as an active endocrine/paracrine organ (reviewed by [44]) has emerged the hypothesis of the pericardial fluid to be, itself, a reservoir of bioactive substances which may regulate heart function and, thus, a relevant material for clinical research [47]. That was, indeed, demonstrated by the trophic and paracrine effects on cultured cardiomyocytes [24, 108]. Only recently have the pericardial exosomes been isolated and their therapeutic properties tested [10, 43], showing that pericardial fluid holds

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great promise in both clinical and biotechnology research fields. Besides, the particular dynamics of pericardial fluid formation and clearance has turned the intrapericardial route one of the most promising delivery methods to treat heart disease. Indeed, apart from drugs and bioactive compounds, stem cells have already been delivered to the pericardial sac [15, 16].

Currently available reviews on the pericardial fluid are globally devoted to the clinical presentation, diagnosis and management of pericardiopathies and their haemodynamic consequences (e.g. [12, 56, 176]). Therefore, our goal is to provide an updated review of pericardial fluid's molecular knowledge, as well as its potential use for diagnostic/prognostic purposes on diseases other than pericardiopathies. To that end, a network mapping the diseases influencing the composition of pericardial fluid is presented, based on an in-depth literature search. Herein, we also give a brief overview on pericardial fluid features and dynamics. A special emphasis is placed on important contributions from pericardial fluid research to basic science, namely by providing mechanistic insights into heart's pathophysiology. We finally discuss the therapeutic potential of the pericardial fluid, particularly the paradigm of intrapericardial administration as a targeted method for drug delivery.

Systematic review of pericardial fluid molecular features

Aiming to collect and analyse all molecular studies on pericardial fluid, exhaustive literature research was performed using the keywords "pericardial fluid". Over 2800 abstracts were retrieved and revised and three levels of exclusion criteria were followed, achieving 88 valid reports for further analysis (the reader is referred to Online Resource 1 for details on the literature research and data mining). Data from the reports were manually curated and organised to extract all molecular entities (e.g. proteins, peptides, enzymes, metabolites and glycans) that could be identified and whose variation has been assessed between two or more conditions (Online Resource 2). A network analysis was then conducted to elicit conditions that have been studied in more detail through pericardial fluid analysis and, more importantly, to evaluate the biomarker potential of the screened molecular species. To that end, an interaction table was created to summarise the variation of those species between different conditions. Only studies reporting significant differences ($p < 0.05$) between two conditions and reporting the direction or degree (fold-change) of the variation were considered. An undirected network was then generated with the Cytoscape [141] program (v.3.6.1), with the conditions defined as nodes and the molecules as edges. The network was tailored so that node size could reflect betweenness centrality and the edge colour the mean decimal logarithm of a molecule fold-change between a node pair, with green meaning the lowest changes ($\log_{10} = 1$) and the red meaning the largest changes ($\log_{10} = 1000$). A small delta was added to indicate, in each pair of pathophysiological settings, where a given molecule is found in higher amounts.

The origin, functions and diagnostic properties of the pericardial fluid

The human heart and the roots of the great vessels diverging from and converging to this organ are surrounded by a protective structure called pericardium (Fig. 1a). Pericardium is a double-walled sac composed of an external layer of connective tissue (fibrous pericardium) and of an internal layer of mesothelial cells (serous pericardium) that coats the inner surface of the fibrous pericardium (parietal layer) and that adheres firmly to the epicardium surface (visceral layer). Apart from the function of a biological barrier to prevent infection, the pericardium, although not essential for life, plays important mechanical, metabolic, immunologic and haemodynamic functions [56, 171]. Enclosed in the pericardium lies a serous, clear and pale-yellow fluid, the pericardial fluid. As compared to plasma, the pericardial fluid presents similar levels of sodium, potassium and chloride, but lower levels of calcium and phosphorus [61] (the reader is referred to Fig. 1b, summarising major biochemistry characteristics of pericardial fluid). Still, its protein concentration is about half of the plasmatic counterpart and albumin occupies a larger fraction (~70%) of the total protein [11, 48]. In physiological conditions, pericardial fluid volume is found in the millilitre range (15–60) [9, 12]. In spite of the low volume, the existence of a fluid surrounding the heart is important to absorb shock and to prevent epicardial friction by providing lubrication and also to equalise transmural pressures during heartbeat [56, 171]. In fact, rabbit pericardium was found to actively secrete hyaluronic acid, which contributes significantly to the resilience and compliance of the pericardial fluid to the permanently experienced compressive and stretching forces [57, 58].

The growing interest on pericardial fluid is explained by its peculiar production and clearance dynamics. Initially, the pericardial fluid was defined as a mere plasma ultrafiltrate. The first evidence was collected by Maurer et al. [107], who demonstrated that pericardial fluid and serum followed Donnan's law for two fluids in equilibrium. This was later corroborated by Gibson and Segal through experiments with rabbits and greyhounds [51]. Indeed, they found that sodium, chloride, calcium and magnesium, but not potassium, ions behaved as expected from a passive plasma ultrafiltrate. However, following findings of Stewart et al. [147] showing that fluid flows from the epicardium to the pericardial sac,

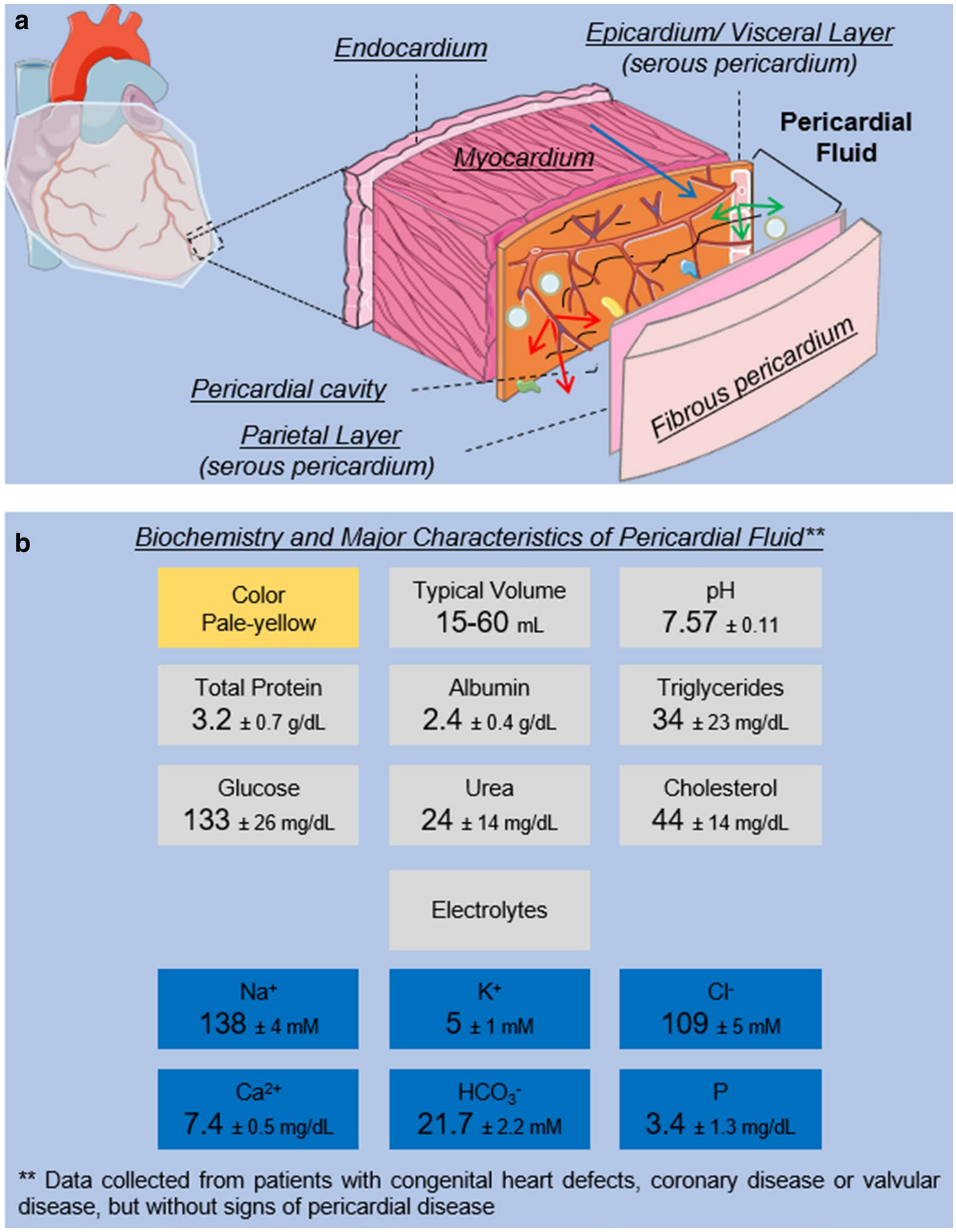

Fig. 1 The Pericardial Fluid ID card. **a** Anatomical representation of pericardium and currently accepted mechanisms of pericardial fluid formation. The pericardial fluid lies in the pericardial cavity and is considered to be the output of plasma ultrafiltration from the epicardial capillaries (red arrows), secretions from pericardial mesothelial cells (green arrows) and contributions of the myocardial interstitial space as a result of hydrostatic and osmotic pressures (blue arrow). **b** Major biochemistry characteristics of the pericardial fluid (data derived from [11, 61])

changed the perspective over pericardial fluid origin. Using a hemispheric capsule firmly attached to the epicardium and filled with albumin in physiological concentrations, followed by measurements of pericardial hydrostatic pressure and flow, the Starling equation was found to govern epicardium-to-pericardium flow. Currently, it is well established that despite the higher epicardial osmotic pressure, the difference between the intrapericardial hydrostatic pressure (lower) and the intramyocardial pressure (higher), drives the flow from the myocardium and epicardium to the pericardial sac [52, 171]. Pericardial fluid is, thus, currently better defined as the net result of plasma ultrafiltration from the epicardial capillary bed (red arrows in Fig. 1a) and possibly from the pericardial parietal layer, but also the product of secretions from the pericardial mesothelial cells (green arrows in Fig. 1a) and contributions of the myocardial interstitial space (blue arrows in Fig. 1a) [10, 56, 171]. Consequently, the pericardial cavity stocks many different bioactive substances [47] such as cardiac hormones [83, 123, 156], growth factors [46, 98, 136], prostaglandins [114] and cytokines [32, 76, 134], whose implications in heart function are still not entirely clear, but may include paracrine regulation of heart contractility, vasodilation and efferent cardiac sympathetic stimulation [114, 165]. These functions are supported by the predominance of biological processes related to the response to stress, stimulus and to wounding, according to the gene ontology enrichment analysis of the pericardial fluid proteins identified so far (Online Resource 1).

Radioactivity tracing studies following intrapericardial delivery of radiolabelled albumin had significantly added to the knowledge of pericardial fluid clearance pathways [52, 96, 150]. Initial experiments supported a higher participation of blood capillaries in the removal of protein from the pericardial sac [150]. However, upon blockage of the major lymphatic ducts (thoracic and right), a concomitant and significant fall in radioactivity traces in blood defined lymphatics as the predominant clearance system [17, 52]. Still, heterogeneity does exist in the disappearance pathways of pericardial fluid constituents. In a series of experiments, both the size and the chemical properties were found determinant for the clearance and systemic distribution of the materials introduced in the pericardial cavity. For instance, a small dye (phenolsulphonephthalein) could be almost completely recovered from human urine (> 82%) 24 h post-intrapericardial administration, while recovery of a larger dye (vital red) was only achieved 1 month after administration [146]. In a leporine model, rapid absorption of Ringer's solution was verified, as opposed to graphite, whose absorption was very slow [27]. Later, India ink's carbon particles delivered to the pericardial sac were detected in the lumen of lymphatic capillaries, but not larger latex particles (0.2 µm in diameter). These were preferably engulfed by macrophages [153]. Cho et al. [21] also showed the inverse relationship between molecular weight and clearance rate (sodium > Cr-EDTA > inulin). In general terms, though, pericardial drainage is considered a slow process. Indeed, while human blood is filtrated at a rate of ~ 120 mL/min [118] (with plasma complete filtration of plasma in less than half hour), complete drainage of sheep's pericardial fluid (~ 8 mL) was estimated to take between 5.4 and 7.2 h [17].

The balance of pericardial fluid formation and clearance can be affected in certain pathological conditions, and accumulation of fluid may ensue. For instance, concomitant pulmonary and systemic venous hypertension resulted in pericardial fluid build-up in canine pericardia [109]. Epicardial transudation was also found to increase with coronary sinus hypertension in dogs [147]. In humans, up to litres of fluid may accumulate, upon inflammation of the pericardium (pericarditis) or physical injury, originating pericardial effusions [82]. Ultimately, this may give rise to life-threatening conditions such as cardiac tamponade, characterised by atrial and pericardial pressure elevation, *pulsus paradoxus* and arterial hypotension [56, 165]. Siding with volume imbalance, molecular changes are also observed in pericardial fluid and both are considered a reflex of heart's pathophysiological status. Currently, though, routine analysis is often limited to biochemical, haematological and cytological/microbiological tests in cases of pericardial effusions. These are generally managed by means of pericardiocentesis and molecular testing is used to inspect the effusion aetiology [12]. Determination of total protein and assessment of lactate dehydrogenase (LDH) are two of the most common biochemical tests applied, as these are required for the classification of transudates and exudates [9, 11]. This classification is essential to uncover the underlying cause of the effusion. For instance, transudative effusions are usually associated with heart failure, hypoalbuminaemia, radiotherapy, cirrhosis and renal insufficiency, while exudative effusions are mainly due to infective, auto-immune or malignant conditions [29, 82]. Other presentations of pericardial fluid include haemopericardium (presence of blood), chylopericardium (chylous appearance, with high levels of triglycerides and lymphocytes), pyopericardium (presence of pus) and pneumopericardium (presence of air) [29]. A culture of pericardial fluid, bacteriological smears and Gram stains are generally ordered when there is suspicion of pericarditis and cytological examinations (such as histological studies and leucocyte counts) are performed upon malignancy imminence [9, 29].

The ethical restraints of collecting pericardial fluid from healthy individuals, requiring an invasive procedure, explain why this is better characterised in conditions manifested as pericardial effusions. However, pericardial fluid can be easily and safely collected during open heart surgery [47]. Moreover, the low turnover rate of pericardial fluid makes this biofluid an appetising source of biomarkers for heart conditions and the pericardium itself a luring reservoir for drug delivery aiming higher residence time and therapeutic efficacy. Thus, in the next sections, we present the results of a systematic review of molecular markers analysed in the pericardial fluid with important diagnostic/prognostic implications for pericardial and non-pericardial diseases. We also discuss mechanistic insights of heart's pathophysiology derived from pericardial fluid basic research as well as the translational potential of intrapericardial drug delivery.

A network view over the molecular signatures of different conditions in pericardial fluid

The network illustrated in Figs. 2 and 3 summarises the current knowledge on pericardial fluid molecular research. Pericardial (Fig. 2) and non-pericardial (Fig. 3) diseases and their phenotypic variations (nodes) are compared regarding the variation of molecular species (edges). Despite the general lack of information in the literature regarding the sensitivity, specificity and positive/negative predictive values, this map reports molecular variations studied so far between different conditions with influence in pericardial fluid composition. Therefore, whether alone or in multiplex, several molecules presented in Figs. 2 and 3 deserve further scrutiny to evaluate their potential as diagnostic/prognostic markers. The colour of the edges represents the degree of variation

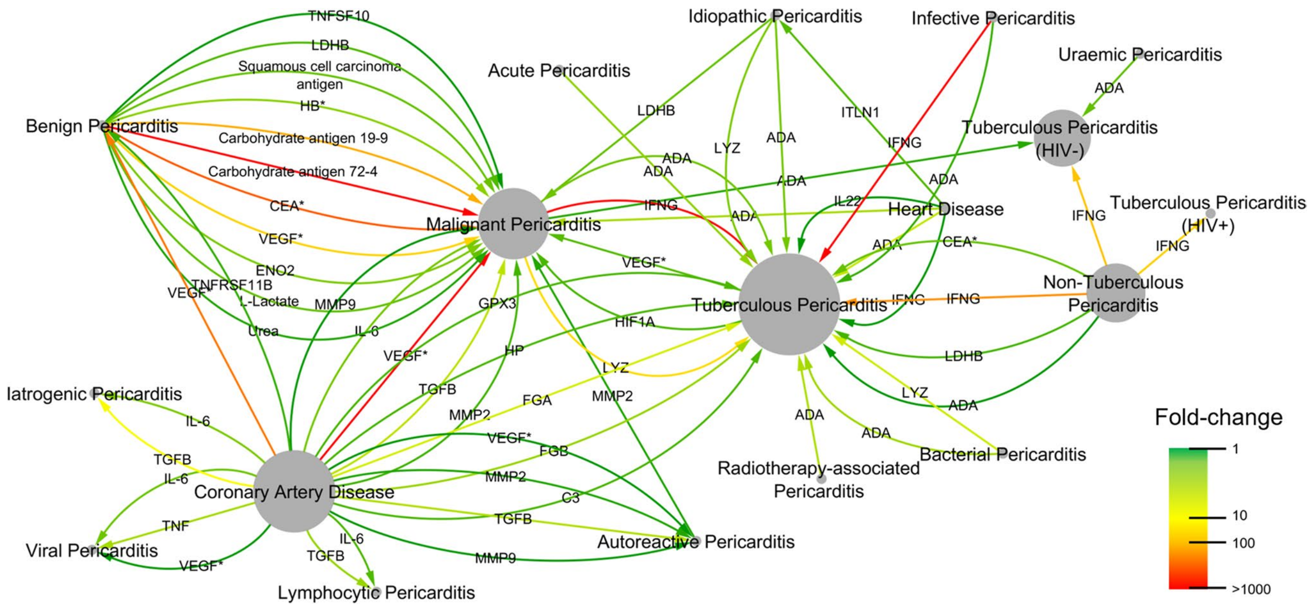

Fig. 2 Current pericardial disease–pericardial disease molecular associations reported in pericardial fluid: a network built using Cytoscape v.3.6.1. Diseases were defined as nodes and the molecular comparisons were represented as edges. Node size represents betweenness centrality (empiric measure of the disease relevance through the number of shortest paths that go through disease to fulfil the entire network). Edge colour translates the mean decimal logarithm of a molecule fold-change: green edges represent less intense variations ($\log_{10}$ closer to 0 ≈ fold-change 1) and red edges represent variations of higher magnitude ($\log_{10}$ closer to 3 ≈ fold-change 1000). Arrows indicate in each pair of diseases, where a given molecule is found in higher amounts. To be readily recognised, proteins are identified with their respective gene name and peptides, glycans and metabolites are given with the respective full name. *HIV* human immunodeficiency virus

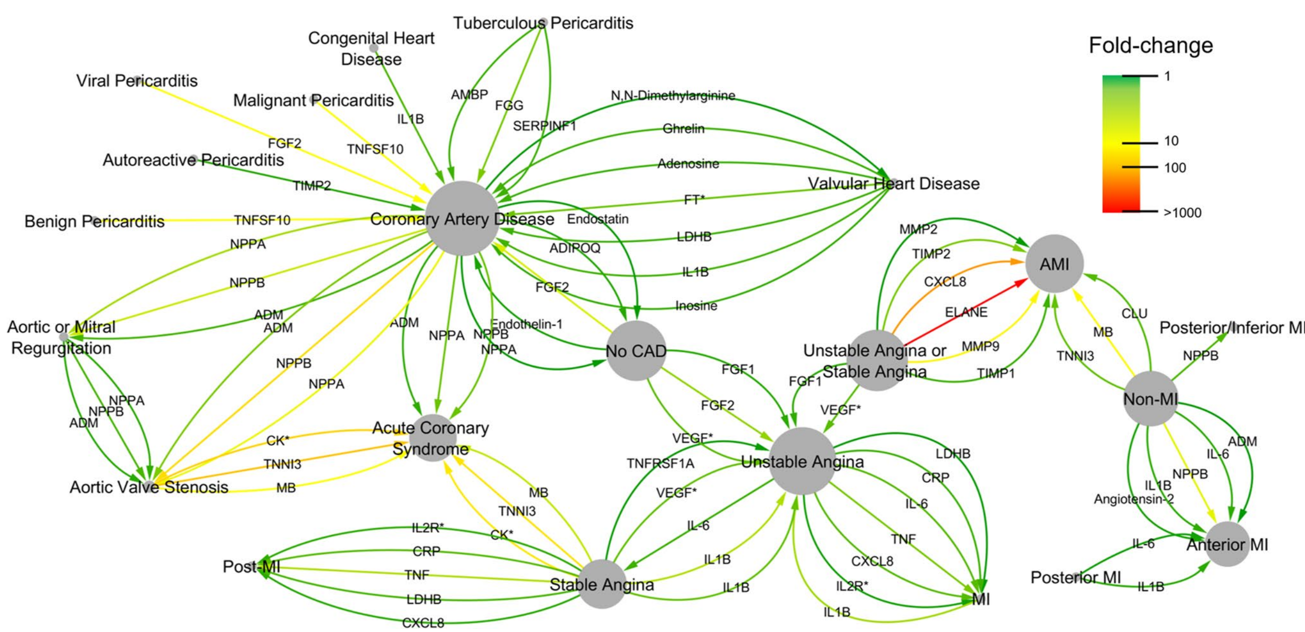

Fig. 3 Current heart disease–heart disease molecular associations reported in pericardial fluid: a network built using Cytoscape v.3.6.1. Diseases were defined as nodes and the molecular comparisons were represented as edges. Node size represents betweenness centrality (empiric measure of the disease relevance through the number of shortest paths that go through a disease to fulfil the entire network). Edge colour translates the mean decimal logarithm of a molecule fold-change: green edges represent less intense variations ($\log_{10}$ closer to 0 ≈ fold-change 1) and red edges represent variations of higher magnitude ($\log_{10}$ closer to 3 ≈ fold-change 1000). Arrows indicate in each pair of diseases, where a given molecule is found in higher amounts. To be readily recognised, proteins are identified with their respective gene name and peptides, glycans and metabolites are given with the respective full name. *AMI* acute myocardial infarction, *MI* myocardial infarction, *CAD* coronary artery disease

of a molecule between a pair of conditions. For instance, the green edge of adrenomedullin (ADM) between coronary artery disease and atrial/mitral regurgitation represents a weaker elevation (2.1-fold) in the latter (in every case, the arrow indicates where the molecule was found in higher amounts). In turn, myoglobin (MB) has shown a medium elevation in acute coronary syndrome as compared to aortic valve stenosis (tenfold higher); thus, this edge is found yellower (Fig. 3). A final example is the stronger elevation (> 1000-fold) of interferon gamma (IFNG) in tuberculous pericarditis versus malignant pericarditis, which is reflected in a redder edge (Fig. 2).

As one may see pericardial fluid molecular research was mainly devoted to conditions directly afflicting the pericardium (tuberculous and malignant pericarditis) or to prevalent heart diseases such as coronary artery disease, valvular disease, angina and myocardial infarction. The remaining network's node pairs related to less explored diseases or phenotypical variations of some conditions can be found in Online Resource 3.

Pericardial diseases influencing the composition of pericardial fluid

Probably one of the first conditions to be studied through pericardial fluid analysis was tuberculous pericarditis. Driven by the difficulty of establishing a definitive diagnosis of *Mycobacterium tuberculosis* infection in patients with strongly suspected tuberculosis and due to the long time required for the culture and identification of the bacilli, researchers have attempted to translate routine analysis of ADA in pleural fluid to the diagnosis of tuberculous pericarditis [3, 79, 139]. Indeed, ADA has been found elevated in the pericardial fluid of patients with tuberculous pericarditis as compared to other conditions such as malignant [25, 78, 81, 95], idiopathic [3, 25, 81, 95] and radiotherapy-associated pericarditis [81] as well as heart disease [79]. Such specificity is probably due to its catalytic activity. ADA promotes the irreversible deamination of adenosine into inosine, which is required for the proliferation and differentiation of lymphocytes that are required to thwart mycobacteria infection [3, 139]. However, it should be noted that it may not be as specific when compared to other conditions manifesting as lymphocytosis, such as sarcoidosis, rheumatoid arthritis and lymphomas [3, 95]. Therefore, besides ADA, other molecules may be helpful in identifying tuberculous pericarditis, such as lysozyme [3, 110], which presents bacteriolytic activity, and IFNG [34, 105, 132], a cytokine released during *M. tuberculosis* infection. This last marker displayed better performance than a qPCR-based method, the Xpert MTB/RIF test, to diagnose tuberculous pericarditis [129].

Similarly to the advances in tuberculous pericarditis research, the diagnosis of malignant pericarditis through the assessment of specific molecules, such as the CEA, has been inspired by observations in pleural and peritoneal fluids [78, 79, 157]. This is probably one of the best established pericardial fluid markers of malignancy as it is generally found over 100 times elevated in subjects with malignant versus benign pericarditis [72, 79, 157] and because in combination with cytological examinations, the assessment of this glycoprotein may achieve 100% of diagnostic sensitivity [157]. Other antigens such as the carbohydrate antigens 19-9, 72-4 and the squamous cell carcinoma antigen have been assessed and compared between patients with malignant and benign pericarditis. However, only the carbohydrate antigen 72-4 has shown enough discriminant power between both conditions [72]. Moreover, matrix metalloproteases 2 and 9 were reported as elevated in patients with malignant pericarditis, which may be explained by the inflammatory burden, but also due to tumour migration [89]. Probably due to the same reason, the vascular endothelial growth factor (VEGF) and the hypoxia-inducible factor-1α, essential factors for angiogenesis, were found in higher amounts in pericardial fluid collected from patients with malignant pericarditis as compared to those with tuberculous pericarditis [99]. Particularly, VEGF was also found increased when compared with populations with autoreactive or viral pericarditis and even with coronary artery disease [73]. Still exploring the inflammation paradigm, researchers have attempted to distinguish malignant pericarditis by assessing the levels of some cytokines. Indeed, the tumour necrosis factor-related apoptosis-inducing ligand (TNFSF10, commonly named TRAIL), was found elevated in malignant pericarditis when compared to benign pericarditis, despite being found in reduced levels when compared to patients with coronary artery disease [71]. Additionally, interleukin-6 and the transforming growth factor β (TGF-β, gene TGFB) have been found in higher amounts in patients with malignancy than in those with coronary artery disease [134]. Despite the assessment of different cytokines and paracrine cues, these analyses require validation due to the limited number of reports available.

Unlike the previously discussed conditions (tuberculous and malignant pericarditis), much less research has been conducted for other pathologies manifesting as pericardial effusions, such as idiopathic, iatrogenic, autoreactive, viral and lymphocytic pericarditis. Still, there are several examples of proteins that were found to be particularly associated with aetiologically distinct pericardial effusions. For instance, the level of omentin-1, an adipokine, was found to be twofold elevated in idiopathic pericarditis as compared to heart disease, through a proteomics-based approach to the analysis of pericardial fluid [180]. TGF-β was also reported as elevated in iatrogenic, autoreactive and lymphocytic pericarditis when compared to coronary artery disease, although it is unspecific, reflecting, perhaps, a regulatory response to overt inflammation and, thus lacking the ability to differentiate between different aetiologies [134]. A final example is the down-regulation of fibroblast growth factor (FGF) 2 in viral pericarditis, also when confronted with coronary artery disease [70], although further studies are required to validate such association.

Non-pericardial diseases influencing the composition of the pericardial fluid

Most of the conditions (pericarditis) discussed previously have in common the fact that they may manifest as effusions, which may threaten life through the development of cardiac tamponade [12, 56]. Often, this medical emergency is resolved by means of pericardiocentesis, which is itself a window of opportunity for the direct exploration of the pericardial sac content. However, in symptomatic patients, pericardiocentesis can be performed for diagnostic purposes only, as there is the need to identify the aetiology of the pericarditis in order to select appropriate treatment [12]. Still, it should be noted that pericardial fluid can be safely and easily collected after pericardiotomy, during open heart surgery [47], and, in some cases, postoperatively, through puncture of pericardial drainage tubes [41], which together increase the chance to look over potential diagnosis/prognosis markers of several heart diseases. As a matter of fact, several proteins and peptides present in pericardial fluid correlate with structural and functional cardiac parameters, while the same markers assessed in plasma or serum do correlate poorly or do not even show significant correlation with the same (the reader is referred to Table 1). Except the BNP, which associated slightly better to left atrial diameter in plasma (0.59) than in pericardial fluid (0.55) [20], or its N-terminal peptide, which showed comparable correlations to left ventricle end-systolic volume and ejection fraction in both fluids [5], the remaining molecules displayed stronger correlations to all the parameters analysed through pericardial fluid analysis. For instance, the pericardial fluid levels of the active (mature) and inactive (glycine-extended) forms of ADM (a vasodilator peptide) [35, 59, 155], the insulin-like growth factor-1 [1], the matrix metalloproteinase-2 [111], required for left ventricle remodelling, and the metabolite asymmetric dimethylarginine [121], an endothelial nitric oxide synthase blocker, showed robust negative correlations to left ventricle ejection fraction, which is of tremendous prognosis value in clinical practice, while the respective plasmatic/serum levels exhibited either weaker or non-significant correlations. Despite the exception of BNP concerning left atrial diameter, more powerful correlations were discovered in pericardial fluid versus plasma/serum in other relevant physiological parameters such as the left ventricle end-systolic and end-diastolic dimensions, right ventricle end-diastolic diameter and the pulmonary artery systolic pressure [20]. In addition to the parameters found in Table 1, it is worth mentioning that there is an inverse relationship of NYHA's classification of heart failure to the concentration of endothelin-1 in pericardial fluid, which is not found in plasma [166]. Furthermore, many of the differences reported between conditions affecting the heart have been exclusively detected in pericardial fluid, while no significant differences were detected when analysing plasma or sera from the same individuals [3, 37, 54, 62, 71, 73, 76, 137, 140, 154, 181] (Table 2). Interestingly almost all markers depicted in this table have a molecular weight below or very close to 40 kDa, which supports the hypothesis that pericardial fluid may accumulate endocardium/myocardium-derived factors [24, 108], which migrate through epicardium into the pericardium sac [127].

Acknowledging all the reasons aforementioned, briefly, the easiness and safeness of collection during heart surgery, the concentration of heart-derived factors and their correlation to echocardiographic and haemodynamic parameters, pericardial fluid should be regarded as a valid and sensitive tool for the diagnosis of heart diseases or even to predict the outcome of patients undergoing cardiac surgery. Indeed, several pericardial fluid molecules could be mapped to many heart diseases, such as coronary artery disease, valvular heart disease and myocardial infarction (Fig. 3). Of note is chronic heart failure that, despite being less represented in the main network, was also associated to increased levels of ANP and the metabolite 3′,5′-cyclic guanosine monophosphate [4] (Online Resource 3).

Coronary artery disease is, perhaps, the most studied heart condition through pericardial fluid analysis. Often do the patients with ischaemic heart disease undergo coronary artery bypass grafting (CABG), which is an excellent opportunity to collect and analyse pericardial fluid. Many proteins have been found to distinguish this from other conditions. For instance, the level of the apoptosis-inducer cytokine TRAIL was found ten times higher in coronary artery disease than in malignant pericarditis [71]. Another cytokine, the interleukin-1-β, a major player in atherosclerosis, was reported to be at least twofold more concentrated in coronary artery disease than in valvular and congenital heart diseases [126]. Growth factors have also been assessed due to their crucial role in coronary collateralisation. Indeed, these factors have proved capable of discriminating coronary artery disease from other well-defined pathologies. For instance, the FGF2 (basic) has drawn much attention because it is essential for coronary collateralisation (angiogenesis) by promoting DNA synthesis and mitosis of both vascular endothelial and smooth muscle cells [181]. Its levels were found to be tenfold higher when compared to patients with viral pericarditis [70] and more than sevenfold higher when compared with patients with no signs of coronary artery disease but with valvular heart disease (aortic or mitral stenosis

Table 1 Comparison of the pericardial fluid (PF) to the plasma/serum (P/S) molecular correlations to structural and functional cardiac parameters described since 2000

Marker	Total ADM		Mature ADM		ADM-Gly		Total IGF-1		Free IGF-1		Total MMP2		Active MMP2		BNP		NT-proBNP		ADMA		Total ghrelin		Acylated ghrelin	
Parameter																								
Biofluid	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S	PF	P/S
LVESV	**0.66**	0.41	**0.63**	0.25	–	–					**0.69**	0.48	**0.64**	n.s.			0.54	**0.55**						
	0.41	n.s.	**0.28**	n.s.	**0.44**	n.s.																		
LVEDV	**0.60**	0.35	**0.60**	0.28	–	–					**0.61**	0.53	**0.56**	n.s.			**0.58**	0.54						
	0.33	n.s.	**0.28**	n.s.	**0.29**	n.s.																		
	0.40	0.33	n.s.	n.s.	**0.48**	n.s.																		
LVEDD															**0.62**	0.56			**0.43**	n.s.				
LVESD															**0.50**	0.43			**0.49**	n.s.				
RVEDD															**0.64**	0.59								
LAD															0.55	**0.59**								
PASP															**0.75**	0.71								
LVDd															**0.63**	n.s.								
PWT																					**−0.35**	n.s.	**−0.31**	n.s.
LVSP	**0.63**	0.50	**0.60**	0.42	**0.56**	0.48																		
LVEDP	**0.44**	n.s.	**0.36**	n.s.	**0.44**	n.s.																		
LVMI	**0.47**	0.38	**0.47**	0.36																				
LVEF	**−0.59**	0.41	**−0.54**	−0.19	–	–	**−0.45**	n.s.	**−0.37**	–	**−0.59**	n.s.	**−0.70**	n.s.	**−0.70**	−0.69	−0.55	−0.55	**−0.45**	n.s.				
	−0.55	−0.31	**−0.36**	−0.34	**−0.59**	−0.31																		
	−0.44	−0.34	**−0.39**	n.s.	**−0.43**	−0.33																		
	−0.32	n.s.	n.s.	n.s.	**−0.33**	n.s.																		

Correlations evaluated in different works are given in different rows. For each study, the best correlation (PF vs. P/S) is marked in bold

ADM adrenomedullin, *IGF-1* insulin-like growth factor 1, *MMP2* matrix metalloproteinase-2, *BNP* brain natriuretic peptide, *ADMA* asymmetric dimethylarginine, *LV* left ventricle, *RV* right ventricle, *LVESV* LV end-systolic volume, *LVEDV* LV end-diastolic volume, *LVEDD* LV end-diastolic diameter, *LVESD* LV end-systolic diameter, *RVEDD* RV end-diastolic diameter, *LAD* left atrial diameter, *PASP* pulmonary artery systolic pressure, *LVDd* LV diastolic dimension, *PWT* posterior wall thickness, *LVSP* LV systolic pressure, *LVEDP* LV end-diastolic pressure, *LVMI* LV mass index, *LVEF* LV ejection fraction, *PF* pericardial fluid, *P/S* plasma/serum

Table 2 Markers that exhibited significant changes between two conditions in pericardial fluid but without significance in blood-derived fluids

Molecule	MW (kDa)	Condition at scope	Compared condition	Fold-change in PF	Refs.
Pericardial diseases					
Adenosine deaminase	40.8	Tuberculous pericarditis	Idiopathic pericarditis	5	[3]
			Malignant pericarditis	18.7	
Lysozyme C	16.5	Tuberculous pericarditis	Idiopathic pericarditis	3.1	
Non-pericardial diseases					
Fibroblast growth factor 1	17.5	Class III unstable angina	Class I/II unstable angina	1.8	[62]
Adenosine	2.7	Coronary artery disease	Valvular heart disease	2.1	[37]
Inosine	2.7	Coronary artery disease	Valvular heart disease	1.9	
Ferritin	20 + 21.2	Coronary artery disease	Valvular heart disease	3	[140]
Fibroblast growth factor 2	30.8	Coronary artery disease	Several (valve stenosis and/or regurgitation; myxoma)	7.4	[181]
Fatty acid-binding protein, heart	14.9	Class III unstable angina	Class I/II unstable angina	1.7	[154]
Ghrelin	3.2	Coronary artery disease	Valvular heart disease	2	[137]
C-type natriuretic peptide	13.2	Left ventricular dysfunction (LVEF < 45%)	Normal left ventricular function (LVEF ≥ 50%)	1.2	[54]
Interleukin-1-beta	30.7	Anterior myocardial infarction	Posterior myocardial infarction	1.7	[76]
Interleukin-6	23.7	Anterior myocardial infarction	Posterior myocardial infarction	1.4	
Non-pericardial vs. pericardial diseases					
TNF ligand superfamily member 10	32.5	Coronary artery disease	Malignant pericarditis	10	[71]
Vascular endothelial growth factor (VEGF165 isoform)	22.3	Coronary artery disease	Malignant pericarditis	0.0004	[73]
Average MW	24.9				

LVEF left ventricle ejection fraction, *MW* molecular weight, *TNF* tumour necrosis factor

or regurgitation) [181]. Beyond the possibility to identify coronary artery disease, which is usually diagnosed before heart surgery, perhaps more interesting is the chance to predict the outcome of patients regarding the coronary collateralisation by pericardial fluid analysis. This hypothesis arises from the observation of increased levels of endostatin, a peptide with anti-angiogenic and pro-apoptotic activities, and of angiostatin, a peptide commonly associated with inhibition of tumour-derived angiogenesis and metastasis, in patients without coronary collateralisation (Grade 0 in Rentrop scale) as opposed to those with high degree of collateralisation (Grade 2–3 in Rentrop scale) [104, 128]. Although, it should be noted that such associations require further validation as, for instance, when categorising patients in Grades 0–1 versus 2–3, endostatin levels were shown to be similar across the groups [98].

Valve replacement surgery, either aortic or mitral, is also a good opportunity for the collection and study of pericardial fluid. Comparisons made between patients with valvular and coronary artery diseases elicited differences at the molecular level, such as the elevation of asymmetric dimethylarginine [121] and the reduction of adenosine, LDH, ferritin and ghrelin in those with valvopathies [28, 137, 140]. Particularly, aortic valve stenosis can be distinguished from ischaemic disease and aortic or mitral regurgitation by an increase in ANP and BNP, in addition to the anti-hypertrophic and anti-fibrotic ADM peptide [123]. In the same study a correlation of the different forms of such peptide (mature, Gly-linked) to some haemodynamic indices (e.g. left ventricle systolic pressure and ejection fraction) was also verified, thus making it an attractive indicator of the severity of heart failure. Therefore, one can hypothesise, that routine analysis of pericardial fluid upon cardiac surgery, may help anticipate the outcome of the patients and, thus, adjust medication to prevent adverse outcomes after valve replacement.

One last example of the research conducted in the pericardial fluid is on angina and myocardial infarction settings. It has been demonstrated that assessment of myocardial injury through pericardial fluid analysis is feasible, which is important for the diagnosis of perioperative myocardial infarction. Despite that, not always the assessment of the classic markers in the pericardial fluid was shown to be better than the assessment in plasma or serum. In fact, while cardiac troponin I performs better in serum to diagnose infarction [38], the determination of the pericardial/serum MB ratio right after admission in the intensive care unit allows its early diagnosis [22]. Curiously, the pericardial cytokine profile differs according to the site of infarction. Indeed, interleukin-6 and interleukin-1-β were found in higher levels in subjects with anterior as compared to those with subsequent myocardial infarction [76]. Additionally, several proteins were shown to distinguish myocardial infarction patients from those with other pathologies. For instance, the C-reactive protein, the matrix metalloproteinases 2 and 9, their tissue inhibitors (1 and 2), the interleukin-8 and the neutrophil elastase were reported to be elevated in patients that have experienced infarction as compared to those with stable or unstable angina [33, 69]. Also, unlike the subjects with aortic root aneurysm or valvular disease, the exosomal clusterin could be identified in the pericardial fluid of patients that have had acute myocardial infarction [43]. Through pericardial fluid analysis, it seems that it is also possible to discriminate between different angina classes (Braunwald's classification). For instance, the FGF1 (acidic) was found 1.8-fold elevated, while the VEGF was reported to be threefold increased between Class III and Class I/II patients [62, 66]. Therefore, even though most patients with unstable angina or suffering an episode of myocardial infarction do not display a tappable pericardial effusion, on occasion of CABG it is possible to collect pericardial fluid and evaluate the performance of markers such as growth factors (e.g. FGF and VEGF) and MB. Their levels might be useful to predict the aggravation of the angina grade or the emergence/recurrence of myocardial infarction episodes.

The use of pericardial fluid as a vehicle for mechanistic studies of heart disease and potential clinical translatability

So far, we have seen that, through pericardial fluid molecular analysis, it is possible to diagnose and prognosticate several conditions directly manifesting in the pericardial cavity or in the heart (Figs. 2, 3), most times with better performance than by plasma or serum analysis (Table 2) and with relevant functional correlations (Table 1). Notwithstanding, exploration of pericardial fluid is not solely helpful for biomarker discovery. Indeed, the pericardial fluid has been used for mechanistic research as well as to address the effects of bioactive compounds, drugs, gene therapy and stem cell therapy on specific pathological conditions (Table 3). Figure 4 provides a snapshot of current pericardial fluid mechanistic research and potential clinical applications, which are discussed in the following sections.

Modulation of coronary blood flow, electrophysiological properties and cardiac inotropy and chronotropy by pericardial fluid

Coronary blood flow regulation has always been a hot topic of research because a tight adjustment of the oxygen and nutrient supply is needed to meet the demand of the cardiac workload. In this sense, the focus has been deposited in understanding if and how coronary blood flow is regulated by molecules present in the pericardial fluid. By artificially increasing afterload (e.g. thoracic aortic constriction) or by β-adrenergic stimulation (e.g. with angiotensin II or isoproterenol), the release of prostacyclin in the canine pericardial cavity has been reported [30, 124, 177]. Soon the role of prostacyclin in the regulation of coronary blood flow was established. By incubating the different cardiac layers with arachidonic acid in vitro, the main source of this hormone was attributed to parietal pericardium in the dog, ox and rat [124]. Beyond prostacyclin, adenosine was also suggested to couple myocardial oxygen consumption with coronary blood flow. The evidence arises from the decrease of pericardial fluid adenosine levels after vagal stimulation (decreasing both parameters) and the increase after atrial constriction, atrial pacing or intravenous delivery of calcium, norepinephrine or isoproterenol (all heightening oxygen demand and, thus, coronary blood flow) [77]. An opposite effect has been described for endothelin-1. Rat carotid arteries incubated with pericardial fluid collected from ischaemic heart disease patients exhibited vasoconstriction and this was prevented by adding an endothelin receptor antagonist [122]. Interestingly, endothelin-1-induced ischaemia is sided by increases in pericardial fluid levels of the vasodilatory adenosine (and its metabolites—inosine and hypoxanthine) [36, 75]. Vice versa, adding adenosine and inosine to canine pericardial cavity led to an increase in endothelin-1 levels [119]. Therefore, vasomodulatory agents present in pericardial fluid regulate coronary blood flow in response to different stimuli. Besides, unlike in plasma, adenosine metabolites and can be more easily monitored in the pericardial fluid due to the relative lower clearance rate [119].

The electrophysiological properties of the cardiac tissue were early hypothesised to be modulated by substances naturally present or added to the pericardial fluid. This was based on the premise that vagal and sympathetic efferent fibres lie at the epicardial surface. Indeed, intrapericardial instillation of tetrodotoxin, prostacyclin and prostaglandin E_2 could rescue the *ansae subclaviae* stimulation-induced shortening of effective refractory period (i.e. the period when an action potential cannot be initiated) [113, 114]. In turn, administration of hexamethonium and tetrodotoxin could mitigate the vagal stimulation-induced lengthening of effective refractory period [113]. Hence, intrapericardial drug delivery may help treat patients with peri- or post-operative arrhythmia. Of note, some pericardial fluid proteins can be themselves arrhythmogenic. For instance, intrapericardial delivery of endothelin-1 induced a significant increase in ST segment, QT interval duration and prolonged the monophasic action potential in dogs. Besides, endothelin-1 caused episodes of ventricular extrasystoles, couplets and triples, tachycardia and fibrillation in a dose-dependent fashion [36, 49, 59, 152, 159].

Table 3 Overview of the studies placing pericardial fluid in the centre piece of basic to translational research

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Pericardial fluid studies aiming for mechanistic insights				
AA, indomethacin[d], isoproterenol (i.v.), angiotensin II (i.v.)	Canine	Epicardial irrigation with drugs Thoracic aortic constriction, carotid occlusion Evaluation of bovine coronary artery tonus (functional detection of prostanoids) RIA [prostanoids]	Most of the prostacyclin present in PF should be of epicardial and/or pericardial origin Prostacyclin should be involved in the regulation of CBF, increasing in conditions of higher workload (pressure overload, β-adrenergic stimulation), where oxygen demand is increased	[30]
AA, isoproterenol (i.v.), angiotensin II (i.a.)	Bovine Canine Murine	Incubation of all animals' pericardia, canine endocardium, myocardium, epicardium and parietal pericardium with AA in vitro Epicardial (canine) irrigation with drugs RIA, HPLC [prostanoids]	All animals' pericardia released prostanoids, enhanced by AA Isoproterenol and AA stimulated the release of 6-oxo-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ into EIF. AA further induced release of thromboxane B_2 and PGE_2 Prostacyclin release was much greater by parietal pericardium, than by epicardium, myocardium or endocardium Angiotensin II stimulated prostacyclin release and this may be a negative feedback response to angiotensin II-induced vasoconstriction	[124] [31]
Calcium (i.v.), norepinephrine (i.v.), isoproterenol (i.v.)	Canine	Induction of atrial constriction, vagal stimulation and atrial pacing Calcium, norepinephrine and isoproterenol administration Analysis of MVO_2 and CBF Spectrophotometry [adenosine]	Pericardial adenosine showed a linear correlation with CBF and MVO_2 Vagal stimulation decreased CBF, MVO_2 and adenosine in PI Aortic constriction, atrial pacing, calcium, norepinephrine and isoproterenol all increased CBF, MVO_2 and adenosine in PI Supports the hypothesis that adenosine couples MVO_2 and CBF and its PF levels may reflect myocardial oxygen demand	[77]
Angiotensin II (i.a.), bradykinin (i.a.)	Canine	Epicardial irrigation with drugs Evaluation of bovine coronary artery tonus Measurement of CBF and BP	Despite the opposite effects in BP and in CBF, both agents induced the release of prostacyclin-like substances in EIF The vasomodulatory activity of PF prostacyclin should be mainly reflected on larger coronary arteries, which are in close contact with PF	[177]
Hexamethonium, tetrodotoxin	Canine	Vagal and *ansae subclaviae* stimulation Electrophysiology/ECG	Vagal stimulation-induced ERP lengthening and *ansae subclaviae*-induced ERP shortening were modulated by the drugs instilled in PF Sympathetic and vagal efferent fibres should be distributed in the epicardium and PF elements should regulate cardiac neurotransmission	[113]
AA, indomethacin, PGE_2, PGI_2	Canine	Vagal and *ansae subclaviae* stimulation Electrophysiology/ECG	*Ansae subclaviae* stimulation-induced ERP, SCL and AH shortening was attenuated by AA, PGI_2 or PGE_2, unless indomethacin was added AA did not avoid vagal stimulation-induced ERP and SCL lengthening PF prostaglandins likely modulate efferent sympathetic stimuli to the heart	[114]
ANP	Canine	Right atrial balloon distension and RV pacing RIA, HPLC [ANP]	Balloon distension and ventricular pacing raised atrial pressure, leading to higher PF ANP levels. ANP correlated with right atrial pressure PF ANP may increase as a response to atrial stretching	[151]
PF collected from patients with IHD, VHD or with Tetralogy of Fallot	Murine	Echocardiography [LV mass determination] Incubation of cardiomyocytes with PF Protein synthesis rate estimation by radioactivity ELISA [bFGF, TGF-β]	PF increased the rate of protein synthesis over serum from the same patients PF's pro-trophic activity was correlated with LV mass PF's trophic effect was abrogated by addition of anti-bFGF antibodies or TGF-β	[24]
PF collected from sheep	Murine	Cell shortening amplitude determination after electrical stimulation	Incubation of adult rat cardiomyocytes with PF induced a potent, rapid and reversible decrease in cell shortening	[108]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Endothelin-1	Canine	ECG	ET-1 administration led to ventricular tachycardia and fibrillation, prolonged QT interval and the duration of endo- and epicardial MAPs	[49]
Endothelin-1	Canine	Haemodynamics and ECG	Haemodynamic variables did not change ET-1 triggered arrhythmias (ventricular extrasystoles, couplets and triplets, ventricular tachycardia and fibrillation) and prolonged QT interval and MAP duration in a dose-dependent manner	[59] [152]
PF collected from patients with unstable or stable angina	Murine	ELISA [ELISA] Incubation of F2 cells (murine vascular endothelial cell line) with PF Cell survival assay (optical density)	VEGF levels were found higher in patients with unstable angina PF from unstable angina patients induced higher levels of cell death, probably by apoptosis (internucleosomal DNA fragmentation was seen)	[64]
PF collected from patients with IHD or without IHD	Human	Incubation of human aortic vascular smooth muscle cells with PF Estimation of cell growth by cell counting	Human vascular smooth muscle cells growth was higher when incubated with PF from patients with IHD	[181]
PF from patients with VHD or with IHD	Murine	Isolation of primary ventricular cardiomyocytes from wild type and $p53^{-/-}$ mice Incubation with 1% PF (with or without catalase) Nucleosomal ladder and TUNEL assay WB [phosphor-ERK1/2, -JNK and -p38 MAPK] ICC [8-hydroxy-2′-deoxyguanosine]	PF from IHD, but not VHD, patients markedly activated p38 MAPK (unless in the presence of catalase) PF from IHD patients induces oxidative stress and activates a p53-independent apoptotic pathway in cardiomyocytes	[65]
Endothelin-1 (i.c.)	Canine	ET-1-induced coronary spastic ischaemia Haemodynamics and ECG HPLC [adenosine, inosine and hypoxanthine] in PF and in plasma	ET-1 induced ischaemia significantly increased the levels of adenosine, inosine and hypoxanthine in the PI but not in plasma The PF, but not the plasma, can reflect the adenosine-mediated metabolic myocardial adaptation to ischaemia	[75]
Endothelin-1	Canine	Haemodynamics and ECG HPLC [adenosine, inosine and hypoxanthine] in PF and in plasma	ET-1 induced significant ST elevations and decreased the epicardial, but not the endocardial, MAP duration as well as the upstroke velocity. Intrapericardial delivery leads to subepicardial ischaemia (due to its vasoconstrictor effect) and stimulates adenosine metabolites release (reflected in PF but not in plasma). Still, preservation of systemic haemodynamic variables suggests that, as opposed to i.c., intrapericardial delivery does not induce transmural ischaemia	[36]
Adenosine, inosine	Canine	Haemodynamics and ECG ELISA [ET-1] in PF and in plasma	Adenosine elicited a negative chronotropic effect and inosine elicited a positive inotropic effect A stepwise increase in ET-1 in PF (but not in plasma) was observed for increasing doses of nucleosides. PF allows to monitor ET-1 and nucleosides exerting influence on the heart	[119]
Endothelin-1	Canine	Haemodynamics and ECG ELISA [ANP, BNP] in PF and in plasma	ET-1 administration did not change haemodynamic variables, but induced a significant ST segment increase ET-1 delivery did not change ANP or BNP plasmatic levels. ANP, but not BNP, increased in PF upon ET-1 infusion	[159]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Dopamine, norepinephrine	Canine	Haemodynamics ELISA [ET-1] in PF and plasma HPLC [adenosine and inosine]	Dopamine and norepinephrine increased HR, BP and LV d*P*/d*T* Catecholamines increased PF, but not plasma, ET-1 and adenosine levels. Inosine remained unchanged in any case	[74]
Angiotensin II	Canine	Haemodynamics ELISA [Big ET-1, ET-1 and ANP] in PF and in plasma	Angiotensin II moderately increase BP and max d*P*/d*T* Angiotensin II increased PF, but not the plasmatic, levels of big ET-1 and ANP. ET-1 levels remained unchanged Conversion of big ET-1 to ET-1 may take longer and occur independently of the observed angiotensin II-induced cardiovascular effects	[160]
PF collected from patients with IHD or with VHD/aortic root aneurysm	Murine	Incubation of HUVECs and mouse embryo stem cells with human PF Evaluation of endothelial cells function (capillary like tube formation) and proliferation (BrdU incorporation) and Nkx2.5 expression PF administration to the pericardium of immuno-deficient mice IHC [Ki67$^+$] in epicardial cells Isolation of human epicardial cells, selection of c-kit$^+$ cells and culture in hypoxic conditions with addition of PF IHC, RT-PCR [WT1 and Tbx18] ELISA [growth factors] in PF	Incubation of HUVECs with either type of PF increased their function, although only PF from IHD patients promoted HUVECs proliferation Incubation of mouse embryo stem cells with PF from IHD patients increased the expression of Nkx2.5 Injection of PF from IHD patients into mice pericardia led to a greater WT1 expression and to a higher proliferation of epicardial cells (Ki67$^+$) Incubation of human-derived epicardial c-kit$^+$ cells with PF from IHD patients in hypoxia, resulted in higher expression of WT1 and Tbx18 Therefore, PF from patients with IHD induces the reactivation of the embryonic gene program of epicardial cells PF levels of HGF, IGF-1 and HMGB1 were higher in patients with ischaemic versus non-ischaemic heart disease, while bFGF was lower	[97]
PF collected from patients with IHD or with VHD	Murine	Isolation of rat carotid arteries Measurement of isometric force	The response curve after PF incubation showed a sustained plateau phase, as compared to KCl treatment, suggesting long-lasting effects PF from patients with IHD present appreciable and durable vasoconstrictor activity, supporting the hypothesis that PF regulates CBF	[122]
PF collected from patients with acute MI or with VHD/aortic root aneurysm Clusterin	Murine	Purification of PF exosomes and proteomics WB, ELISA [clusterin] LAD-ligation induced MI and isolation of epicardial cells Haemodynamics Isolation of epicardial cells IHC [clusterin] and histological assessment of EMT, vascularity and apoptosis (TUNEL)	Clusterin was found in higher levels in exosomes purified from PF collected from MI patients Clusterin treatment improved haemodynamics (reduced LV end diastolic pressure and higher min and max d*P*/d*T*) Mice treated with clusterin showed higher levels of epicardial cells undergoing EMT, reduced apoptotic rate and enhanced arteriolar length density	[43]
PF collected from patients with VHD	Human Murine	Purification of PF and plasma exosomes and miRNA array Incubation of HUVECs with exosomes Evaluation of apoptosis (caspases), proliferation (BrdU) and angiogenesis (network formation) Left femoral artery-induced limb ischaemia Intramuscular delivery of the exosomes Histological assessment of capillary density Assessment of blood flow by Doppler imaging	HUVECs treated with PF exosomes showed reduced apoptosis, increased proliferation and increased angiogenic capacity. When treated with plasma exosomes, none of the effects were verified The miR let-7b-5p found in exosomes showed angiogenic activity Mice treated with PF-derived exosomes presented with improved post-ischaemic blood flow recovery, reduced incidence of toe necrosis and greater capillary density. This was not verified when mice were treated with plasma-derived exosomes	[10]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Effects of bioactive compounds and drugs on specific pathological contexts				
AA, indomethacin	Canine	Ischaemia/reperfusion injury induced by temporary LAD ligation *Ansae subclaviae* stimulation Radioactivity [myocardial perfusion evaluation] ECG	AA reduced significantly ventricular fibrillation during reperfusion and mitigated *ansae subclaviae* stimulation-induced rise in BP (unless when indomethacin was added) AA delivery did not affect myocardial perfusion in either ischaemic or non-ischaemic areas	[115]
bFGF	Leporine	i.v. angiotensin II-induced CMI Histological assessment of capillary density, fibrosis and necrosis	Angiotensin II induced LV hypertrophy and fibrosis in rabbits Intrapericardial delivery of bFGF increased angiogenesis score	[91]
bFGF, heparin sulphate	Canine	Inorganic mercury-induced acute MI Analysis of the EF Histology, IHC [α-smooth muscle actin] for determination of capillary and arteriolar density and infarct size	bFGF administration ameliorated EF, 1 month after MI Dogs treated with bFGF and heparin sulphate presented the lowest weight of infarcted zone in relation to total LV weight and the highest density of capillaries and arterioles (stimulation of angiogenesis), especially in the outer halves of the heart	[167]
bFGF, heparin sulphate	Swine	LCX constriction-induced CMI Haemodynamics and MRI Determination of collateral index Histological assessment of vascularity	bFGF improved collateral index and LCX CBF, 4 weeks after injection bFGF did not improve EF but ameliorated LCX regional wall thickening, myocardial perfusion as well as the ischaemic zone % in a dose-dependent fashion	[87]
Paclitaxel	Swine	Injury of the LAD and LCX by balloon distension (angioplasty) Haemodynamics and ECG Histological assessment of apoptosis Vessels morphometry	Intrapericardial delivery was well tolerated, but induced adhesions Higher degree of neointimal cells apoptosis was found for animals treated with the higher dose Paclitaxel reduced neointimal area as well as maximal intimal and adventitial thickness. It induced a positive remodelling effect, demonstrated by reduced degree of luminal occlusion, showing an antirestenotic effect	[60]
^{131}I-labelled diazeniumdiolated bovine serum albumin	Swine	Injury of the LAD and LCX by balloon distension (angioplasty) Haemodynamics and ECG Vessels morphometry	Percutaneous intrapericardial drug delivery was well tolerated NO-releasing drug reduced significantly neointimal and adventitial area and thickness after balloon injury in a dose-dependent manner The drug also induced a positive remodelling effect, translated in increased vessels luminal area, showing promise as a potential antirestenotic agent	[8]
^{125}I-labelled bFGF	Swine	LCX constriction-induced CMI Radioactivity measurement in heart, lung, liver, kidney, plasma and PF	bFGF delivery did not cause haemodynamic changes or complications Minimal deposition of bFGF was found in lungs, liver, kidney and plasma. Myocardium, pericardium and PF concentrated the highest amount of bFGF. bFGF was found 18.5-fold higher in ischaemic versus control animals Epicardial was 10- to 100-fold higher than endocardial radioactivity, reflecting poor transmyocardial diffusion	[88]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Ibutilide	Canine	Atrial pacing-induced AF Haemodynamics Electrophysiology/ECG HPLC [ibutilide] in PF, plasma and cardiac tissue	Ibutilide increased AF cycle length, but it did not increase QT interval and RV MAP over control nor did it show a higher AF termination rate Ibutilide concentration was greater in PF than in plasma, greater in atria than in ventricles and tended to decrease from epicardium to endocardium Despite the atrial electrophysiological effect, ibutilide did not terminate AF	[170]
Suc-Val-Pro-Phe[p] (chymase inhibitor)	Canine	Epicardial abrading for 200 times and analysis of chymase activity in LV 1-2 months after surgery ELISA [TGF-β1] in PI Determination of adhesion areas and histology	TGF-β1 levels in PF did not increase, adhesion area and mast cell density were lower in dogs treated with chymase inhibitor Chymase inhibitor may prevent post-surgical adhesions in humans	[143]
DHA	Swine	Ischaemia/reperfusion injury induced by temporary occlusion of the LAD Haemodynamics and ECG Histological assessment of infarct size	DHA prevented HR increase during ischaemia Arrhythmia scores were diminished during ischaemia and during the first 9 min of reperfusion in DHA-treated animals DHA reduced number of pigs showing ventricular fibrillation, reduced infarct size and prevented mortality	[179]
Amiodarone	Canine	Atrial pacing-induced AF Haemodynamics and ECG HPLC [amiodarone] in the cardiac tissue	Amiodarone increased SCL in a dose-dependent manner. Except for endocardial LV ERP, all ERPs measured were increased by amiodarone Amiodarone in the highest dose significantly reduced stable AF events Drug concentration reduced from outer- (sinus node and atria) to innermost tissues (LV endocardium and septum) and was neglectable in sera	[7]
Comparison of intrapericardial with other administration routes				
Sodium nitroprusside	Canine	LAD endothelial injury and stenosis induction by gentle squeezing and constriction[f] Intrapericardial or i.v. delivery of the drug Haemodynamics Evaluation of platelet aggregation ex vivo	Frequency of CBF variation was significantly lower by intrapericardial administration than by i.v. at the same doses The drug reduced mean aortic pressure and peripheral resistance, but the magnitude was significantly higher when given i.v. (systemic effects) Intrapericardial delivery reduced platelet aggregation more dramatically	[175]
^{125}I-labelled bFGF	Canine	Delivery by i.v., left atrial, pulmonary artery[g], i.c. or by intrapericardial injections Radioactivity measurement in heart, lung, liver, spleen and kidney	Intrapericardial delivery resulted in the highest bFGF deposition in the heart (19% versus 3-5% i.c., 1.3% left atrium, 0.5% i.v. and pulmonary artery) and in a 100-fold increase in heart/lung ratio as compared to i.v. The bFGF epicardium-to-endocardium ratio was about 10 times	[93]
Nitroglycerin	Swine	Intrapericardial or i.c. injection Haemodynamics and ECG Ultrasound-mediated measurement of coronary cross-sectional luminal area	i.c., but not intrapericardial, injection promoted a transitory increase in HR and a decrease in BP Intrapericardial delivery led to a higher and more sustained increase in luminal area	[173]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Radiolabelled bFGF, platelet-derived growth factor, albumin, NO-releasing albumin diazeniumdiolate	Swine	Intrapericardial or endoluminal delivery Autoradiography [LAD and LCX arteries] Determination of the fractional intramural delivery Determination of NO-releasing albumin's redistribution rate by serial gamma imaging	Intrapericardial delivery was feasible and safe Fractional intramural delivery and arterial tissue retention was extremely variable and less reproducible by the endoluminal route Redistribution rate is substantially more rapid through endoluminal release Drugs half-life times increased through intrapericardial delivery Pericardial delivery is advantageous over endoluminal drug delivery	[148]
Fluorescently labelled heparin, albumin, bFGF, IgG, cortisol Radiolabelled bFGF	Murine	Acute or continuous (7 days) intrapericardial or intraarterial administration of the proteins Fluorescence measurement in plasma and PF Autoradiography [cardiac tissue]	Intrapericardial delivery always resulted in higher concentration of heparin, albumin, bFGF, IgG and cortisol in PF and in higher bFGF cardiac tissue penetration than intraarterial administration Pericardial clearance of heparin, albumin, bFGF and IgG was much slower (10.6- to 83-fold) than plasma clearance Pericardial delivery is advantageous over systemic drug delivery	[55]
Procainamide	Swine	Intrapericardial or i.v. delivery Electrophysiology Determination of PF and plasma procainamide levels	Intrapericardial administration increased atrial ERP more sustainably Both routes prolonged interatrial conduction times at the highest doses and increased atrial (but not ventricular) fibrillation thresholds i.v. but not intrapericardial delivery prolonged AV and interventricular conduction times RV MAP duration were significantly reduced after intrapericardial administration (high dose), but not by i.v. Intrapericardial administration is safe and effective and does not affect global ventricular electrophysiology, despite the lower half-life in PF	[168]
Nitroglycerin	Swine	LAD occlusion-induced myocardial ischaemia Dobutamine i.c. infusion Intrapericardial and i.v. drug administration Haemodynamics and ECG	Intrapericardial delivery blocked ischaemia-induced ventricular fibrillation The magnitude of ischaemia-induced T-wave alternans decreased 45 min after intrapericardial injection, but recovered 75 min after Dobutamine-induced increase in dP/dT max (but not HR) was mitigated by nitroglycerin administration (if dobutamine was given not after 15 min) Intrapericardial delivery resulted in smoother BP and dP/dT max decrease than i.v. delivery. Both promoted a transient HR rise, but the former is safer	[84]
Sotalol, atenolol	Murine	Intrapericardial or i.v. delivery HPLC [drugs] in PF, plasma and cardiac tissue Tachycardia induction by i.v. nitroprusside, dobutamine infusion and haemodynamics	Cardiac tissue sotalol and atenolol levels were, respectively, 3.8-fold and 4.7-fold higher after intrapericardial as compared to i.v. delivery Mitigation of nitroprusside-induced increase in HR and of dobutamine-induced increase in dP/dT max was greater through intrapericardial delivery	[18]
IGF-1	Ovine	Intrapericardial or subcutaneous delivery LAD or LCX occlusion-induced heart failure Analysis of the EF	Intrapericardial, but not subcutaneous, administration of IGF-1 increased EF after infarction induction 14 days after treatment withdrawal, EF remained higher than in controls	[106]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Metoprolol	Swine	Intrapericardial or i.v. delivery Isoproterenol-induced sinus tachycardia AF induction Haemodynamics and ECG	Only intrapericardial delivery decreased HR upon tachycardia induction i.v., but not the intrapericardial, delivery reduced d*P*/d*T* and BP Neither delivery route has tackled ECG changes after AF induction Metoprolol half-life time was higher when given intrapericardially Intrapericardial delivery is best suited for tachycardia than for AF treatment	[133]
Digoxin, procainamide	Swine	Intrapericardial (chronic) and i.v. delivery ECG Histological assessment of inflammation and fibrosis	None of the animals showed atrial or ventricular arrhythmias Intrapericardial, but not the i.v., delivery of digoxin and procainamide decreased and increased the corrected QT interval, respectively i.v., but not the intrapericardial, delivery decreased the RR interval. Though, only the later has increased QRS duration significantly Animals showed moderate inflammation and fibrosis in the areas adjacent to the catheter at 6 months, which may preclude translation to humans	[80]
Pursuing gene therapy through intrapericardial delivery				
HVJ encoding β-galactosidase, luciferase and ACE[h] formulated as a liposome complex	Murine	Direct i.m., intrapericardial and i.c. injection of the viruses Analysis of gene expression at necropsy: Histology [β-galactosidase]	β-galactosidase expression was limited to injection sites through i.m., was widespread but inconsistent (only ~ half of the animals showed staining) by i.c. injection, but was widespread and consistent by intrapericardial delivery (covering the surface myocardial layers beneath pericardium, either in cardiomyocytes or fibroblasts) i.m. injection was associated with fibrosis, while i.c. or intrapericardial routes were not associated with toxicity or inflammation	[6]
Adenovirus encoding β-galactosidase, doxycycline	Canine	Delivery of the adenovirus with or without doxycycline Histology [β-galactosidase] Luminometry [β-galactosidase]	β-Galactosidase expression was found in the parietal pericardium, in localised areas of the visceral pericardium, LV myocardium, left and right atrial appendages and in some epicardial arterioles and venules Addition of doxycycline increased β-galactosidase expression, increased activity in epicardium and decreased in parietal pericardium, probably through increased visceral pericardium permeability	[90]
Adenoviruses encoding β-galactosidase and luciferase	Murine	Analysis of gene expression at necropsy: Luminometry [luciferase] Histology [β-galactosidase]	Luciferase expression was much bigger in the heart, than in lungs or liver β-Galactosidase expression was confined to epicardium, myocardium outer half and RV. Additional expression in parietal pericardium, interventricular septum, LV, atrial appendages, endocardium, valves, coronaries and liver was observed by injecting double dose, but this resulted in higher mortality	[183]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Adenoviruses encoding β-galactosidase, luciferase and human α_1-antitrypsin	Canine	Analysis of gene expression at necropsy: Histology [β-galactosidase] Luminometry [luciferase] Radial immunodiffusion assay [α_1-antitrypsin] in PF ECG	Animals tolerated therapy well and did not show ECG alterations β-Galactosidase expression was diffuse in visceral and parietal pericardium Luciferase expression was greater at the pericardium, less pronounced at the LV epicardium and neglectable in endocardium, spleen, lung or liver Human α_1-antitrypsin was appreciable in PF, but not detected in serum The pericardial sac may be used as a sustained-release drug delivery system	[101]
Adenoviruses encoding β-galactosidase and $VEGF_{165}$	Canine	LCX constriction-induced CMI Histology [β-galactosidase] and assessment of myocardial fibrosis, inflammation and vascularity ELISA [VEGF] in PF and serum Measurement of myocardial perfusion[i] by fluorescence	β-Galactosidase expression was limited to epicardium, parietal pericardium and scantly on mid-myocardium. Peripheric expression was negative VEGF levels increased in PF and reduced gradually with a response duration of about 2 weeks. Serum VEGF levels did not increase Myocardial perfusion, degree of vascularity, levels of inflammation and fibrosis were all similar across the groups All treated dogs displayed pericardial effusions	[94]
Adenoviruses encoding HGF	Murine	LAD ligation-induced MI Single- or repeated injections of the virus MRI for the evaluation of cardiac structure and function WB [HGF] Histological assessment of angiogenesis	Higher expression of HGF was found in the peri-infarct areas in animals treated with repeated versus those treated with single injection 12 weeks post-infarction, animals treated with repeated injections showed better LV EF than those treated with a single injection Capillary density was higher in animals treated with repeated injections when compared to those treated with a single injection	[172]
Stem cell therapy by intrapericardial administration[j]				
Bone marrow-derived mononuclear cells	Swine	LAD occlusion-induced MI Immunohistochemical determination of stem cells homing (Hoechst)	Stem cell delivery was not associated with complications (perforation, arrhythmias, haemodynamics disturbances…) Large penetration of stem cells was found in the infarcted areas of the myocardium 21 days post-infarction and concomitant cell delivery	[19]
Gelfoam-enable bone marrow-derived mesenchymal stem cells (autologous)	Swine	LAD occlusion-induced MI Immunocytochemical detection of stem cells PCR confirmation Echocardiography	Stem cell delivery encased in gelfoam was safe and reliable No pericardial effusions developed up to 1 month after treatment Intravascular ultrasound guidance is minimally invasive and provides the safety necessary for percutaneous intrapericardial stem cell delivery MSCs were successfully engrafted as confirmed 1 week after delivery	[86]
Bone marrow-derived mesenchymal stem cells	Swine	Incubation of MSCs with PF Determination of cell viability, proliferation rate and cell surface markers by flow cytometry LAD occlusion-induced MI MRI, histology, Y chromosome amplification [MSCs' distribution]	Viability, proliferation rate and phenotype were not affected by incubation of MSCs with PF MSCs were found more restricted to infarcted areas of the LV (reaching the inner layers) in infarcted animals and were more diffusive in sham animals MSCs reached LV, RV and right atrium and persisted up to 7 days Stem cell administration by the intrapericardial route is safe and feasible	[16]

Table 3 (continued)

Agent[a]	Animal model	Methods[b]	Main findings[c]	Refs.
Cardiosphere-derived cells	Swine	LAD occlusion-induced MI MRI for the evaluation of cardiac structure and function Characterisation of the PFs and plasmatic cellular fraction by flow cytometry, biochemistry analytes and cytokine profile by multiplex immunoassay	CDCs delivery did not result in adverse side effects nor cardiotoxicity 30 days post-administration, CDCs did not change $CD8^+$ T cells or NK cells, but induced an increase in $CD4^+$ T cells and a decrease in $CD8^+CD16^+$ T cells[k] in PF, corroborating CDCs' immunomodulatory properties GGT and interferon α were found decreased in PF after CDCs delivery Although, no differences were found in any of the cardiac structure and function parameters after CDC administration, probably due to the time frame between infarction and study end (7 weeks)	[15]

AA arachidonic acid, *ACE* angiotensin converting enzyme, *AF* atrial fibrillation, *ANP* atrial natriuretic peptide, *bFGF* basic fibroblast growth factor, *BNP* brain natriuretic peptide, *BP* blood pressure, *CBF* coronary blood flow, *CDCs* cardiosphere-derived cells, *CMI* chronic myocardial ischaemia, *CO* cardiac output, *DHA* docosahexaenoic acid, *dP/dT* rate of pressure development, *EF* ejection fraction, *EIF* epicardial irrigation fluid, *ERP* effective refractory period, *ET* endothelin, *GGT* γ glutamyl transferase, *HGF* hepatocyte growth factor, *HMGB1* high mobility group box protein 1, *HPLC* high performance liquid chromatography, *HR* heart rate, *HVJ* haemagglutinating virus of Japan, *HUVECs* human umbilical vein endothelial cells, *ICC* immunocytochemistry, *IGF-1* insulin-like growth factor 1, *IHC* immunohistochemistry, *IHD* ischaemic heart disease, *i.a.* intraaortic, *i.c.* intracoronary, *i.m.* intramyocardial, *i.v.* intravenous, *LAD* left anterior descending artery, *LCX* left circumflex artery, *LV* left ventricle, *MAP* monophasic action potential, *MI* myocardial infarction, *MRI* magnetic resonance imaging, *MSCs* mesenchymal stem cells, MVO_2 myocardial oxygen consumption, *PF* pericardial fluid, *PG* prostaglandin, *RIA* radioimmunoassay, *RV* right ventricle, *SCL* sinus cycle length, *Tbx18* T-box transcription factor 18, *TUNEL* terminal deoxynucleotidyl transfer-mediated end labelling, *VEGF* vascular endothelial growth factor, *VHD* valvular heart disease, *WB* western blot, *WT1* Wilms tumour 1

[a]Intrapericardial administration, unless stated otherwise: i.a.: intraaortic; i.c.: intracoronary; i.v.: intravenous

[b]Main techniques are indicated, assessed parameters given in brackets

[c]Effects of pericardial fluid itself or reflected in this biofluid are described. Epicardial irrigation fluid (EIF) and pericardial infusates (PI) were considered artificial variations of pericardial fluid but, in any case, reflected the fluid collected from the pericardial cavity

[d]Cyclooxygenase inhibitor

[e]6-oxo-$PGF_{1\alpha}$ and PGE_2

[f]Promotion of platelet aggregation and dislodgement with alteration of coronary blood flow

[g]Injection through Swan–Ganz catheter

[h]Luciferase and angiotensin converting enzyme (ACE) were only used to assess the efficiency of the new formulation method with the "naked" plasmid DNA

[i]Assessed by the ratio of coronary conductance between the collateral and the normal zone

[j]Direct epicardial application not considered

[k]$CD8^+CD16^+$ T cells are considered to be terminally differentiated effector T cells

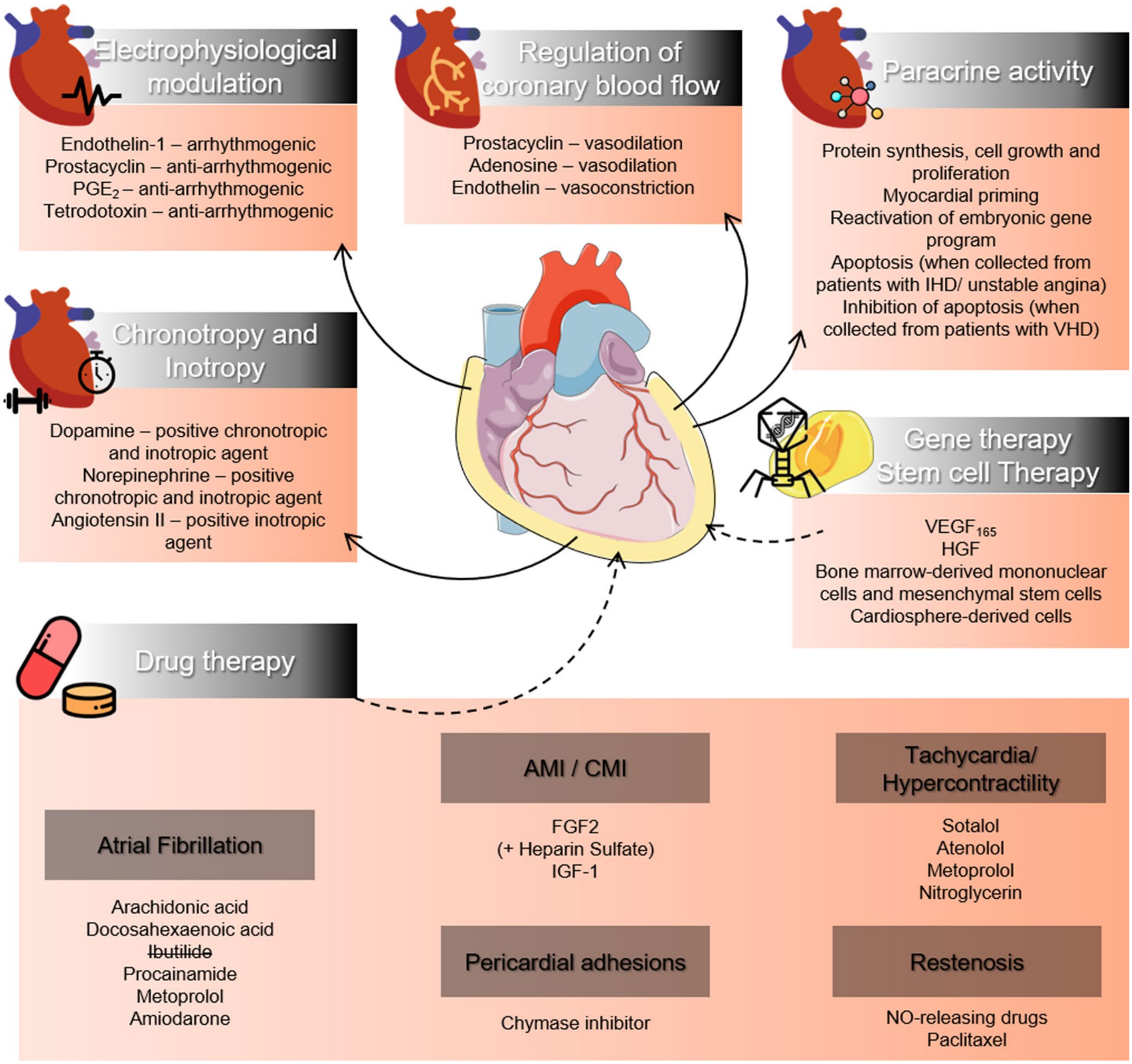

Fig. 4 Summary of the mechanistic research conducted in pericardial fluid (solid arrows) and of intrapericardial therapeutics tested in animal models (dashed arrows). Some graphical elements were adapted from Servier Medical Art repository (http://smart.servier.com/) and Flaticon website (www.flaticon.com). *AMI* acute myocardial infarction, *CMI* chronic myocardial ischaemia, *HGF* hepatocyte growth factor, *IGF-1* insulin-like growth factor, *IHD* ischemic heart disease, *NO* nitric oxide, *PGE*$_2$ prostaglandin E_2, *VEGF* vascular endothelial growth factor, *VHD* valvular heart disease

Administration of inotropic and chronotropic agents to the heart can be life-saving in conditions requiring urgent correction of haemodynamic imbalances such as in cardiogenic shock [120]. In this regard, the intrapericardial route is effective. For instance, intrapericardial delivery of dopamine and norepinephrine in a canine model translated into increased chronotropy and inotropy as demonstrated by, respectively, increased heart rate and LV contractile force (dP/dT) [74]. In turn, angiotensin II induced a rise in the vasoconstrictor endothelin-1 and increased maximum rate of pressure development (dP/dT) in dogs [160].

Exploration of pericardial fluid's paracrine activity

Since the first assessment of growth factors, in 1996, the pericardial fluid has been regarded not only as of diagnostic value, but also as a functional liquid with important paracrine properties. In order to explore these features, mechanistic research has been conducted by incubating specific cardiac and vascular cells with pericardial fluid collected from different animals and human with different pathological conditions. For instance, Yoneda et al. [181] demonstrated that the incubation of human aortic smooth muscle cells with 10% of pericardial fluid collected from patients with ischaemic heart disease could accelerate their growth as compared to that retrieved from nonischaemic patients. Such an effect was, in part, attributed to the higher content in FGF2 in ischaemic patients. The same factor was also found responsible for the stimulation of protein synthesis in cultured adult rat cardiomyocytes after incubation with pericardial fluid collected from patients with ischaemic, valvular or congenital heart diseases [24]. Pericardial fluid from ischaemic heart disease patients also induced beneficial effects in human umbilical vein endothelial cells and in mouse embryo stem cells. While the former presented higher ability of capillary-like tube formation and increased proliferative activity, the latter showed augmented expression of the cardiac marker Nkx2.5 [97]. Perhaps more importantly, the addition of the ischaemia-conditioned pericardial fluid to the pericardial sac of immunodeficient mice boosted the reactivation of the epicardial cells' embryonic gene program [97].

Despite these apparent positive effects on cells, caution should be given to the biofluid source because its contents may induce disparate effects according to the species, the pathological background and the cell type tested. For instance, fluid collected from sheep pericardial sac was shown to decrease the amplitude of myocyte shortening and to increase the resting sarcomere length, through a calcium-independent effect [108]. Also, pericardial fluid obtained from subjects with different conditions exhibited opposite effects in cultured endothelial cells: while fluid from patients with unstable angina promoted apoptosis [64], fluid (exosomes) collected from patients with valvular heart disease inhibited [10]. In primary mouse ventricular cardiomyocytes, fluid from ischaemic heart disease subjects also induced apoptosis. In this case, an oxidative stress-sensitive p38 MAPK cascade was suggested as responsible for cell death activation [65].

Intrapericardial drug delivery as a promising route for the treatment of heart disease

Previous observations of prolonged rates of absorption from the pericardial sac and the acknowledgment of this body cavity's tightness (no leakage observed during epicardial superfusion) [113] have prompted the investigation of pericardial fluid drug instillation for the treatment of heart diseases. Examples include ischaemia/reperfusion injury, acute myocardial infarction, chronic myocardial ischaemia and fibrillation.

One major concern after ischaemia/reperfusion injury is the increased risk of arrhythmia and fibrillation [40]. After acute coronary occlusion-induced efferent sympathetic stimulation, the risk of ventricular arrhythmias is increased. Hence, the regulatory role of some bioactive agents has been tested to prevent arrhythmogenesis. For instance, intrapericardial administration of arachidonic acid in a canine model did not ameliorate myocardial perfusion, but reduced significantly ventricular fibrillation, during the reperfusion phase after LAD ligation [115]. Another polyunsaturated fatty acid, the docosahexaenoic acid, was found to mitigate ventricular fibrillation, reduce infarct size and prevent mortality in a swine model of ischaemia/reperfusion [179]. In models of isolated atrial fibrillation, provoked by atrial pacing, not all drugs have shown efficacy. While ibutilide failed to terminate atrial fibrillation [170], amiodarone was efficacious [7]. Increased sinus cycle length and reduced number of atrial fibrillation events were among the effects of intrapericardially instilled amiodarone [7]. Of note, diffusion, and thus, the potency of these drugs is lost from the epicardium to endocardium and from atria to ventricles [7, 170]. For instance, amiodarone prolonged all except the endocardial left ventricle effective refractory period, probably due to the thicker wall [7]. Therefore, when translating these treatments to the clinical setting, pericardial residence time should be tailored (e.g. by adjusting concentration) so that drugs can reach deeper zones of the endocardium.

Intrapericardial delivery of FGF2 has also evidenced high potential for the treatment of acute myocardial infarction and chronic myocardial ischaemia. In a canine model of acute myocardial infarction, FGF2 improved ejection fraction 1 month after infarction [167]. Furthermore, dogs treated simultaneously with FGF2 and heparin sulphate showed the lowest infarct weight and the highest capillary and arteriolar density, over those treated with FGF2 alone [167]. In turn, in a leporine model of angiotensin II-induced chronic ischaemia, FGF2 augmented the angiogenesis score [91]. Finally, in a swine model of LCX constriction-induced chronic ischaemia, FGF2 improved collateral index and LCX blood flow, 4 weeks post-intrapericardial injection [87]. In the same model, high amounts of FGF2 were found in myocardium, pericardium and pericardial fluid and residual traces in lungs, liver, kidney and plasma, showing the specificity accomplished by intrapericardial delivery [88].

Beyond the usefulness of the intrapericardial delivery route to tackle heart disease, per se, this method can also be important in alleviating secondary effects of cardiac interventions. For instance, LAD and LCX angioplasty is known to cause injury to the cardiac vessels, inducing restenosis. Nonetheless, the nitric oxide-releasing diazeniumdiolated bovine serum albumin and paclitaxel were found to mitigate its secondary effects, as evidenced by increased vessel luminal area (positive remodelling effect) [8, 60]. Another common finding after cardiac surgery is pericardial adhesions. One mechanism explaining the development of adhesions is the TGF-β-induced fibrosis, with TGF-β being released from extracellular matrix due to the activity of proteases such as chymase [45]. In this sense, a chymase inhibitor was injected into the pericardial cavity in dogs, after epicardial abrading 200 times. Successfully, this inhibitor reduced adhesion areas and mast cell density in treated dogs [143]. Therefore, intrapericardial delivery of chymase inhibitor may be used to prevent post-surgical adhesions in humans.

The studies discussed hitherto have demonstrated satisfactory effects in treating a myriad of heart conditions. Notwithstanding, before minding clinical translation, comparison with different administration routes in animal models is imperative. Several studies (Table 3) have demonstrated the advantages of intrapericardial delivery. In what regards deposition in cardiac tissue, intrapericardial injection of FGF2 showed the highest percentage of heart deposition (19%), as compared to intracoronary (3–5%), left atrium (1.3%), intravenous (0.5%) and pulmonary artery injections (0.5%) [93]. When comparing to intraarterial administration, FGF2 cardiac tissue penetration was eightfold higher [55]. Other drugs such as sotalol and atenolol reached higher levels in cardiac tissue (3.8- and 4.7-fold, respectively) by intrapericardial than by intravenous delivery [18]. Intrapericardial delivery further bypasses the extremely high variability of fractional intramural delivery attained by the endoluminal route [148]. This is explained by the longer pericardial residence time achieved for the tested compounds (radiolabelled FGF2, platelet-derived growth factor and albumin) and corroborated by lower clearance rates of these [148] and other drugs, like heparin [55] and metoprolol [133]. Importantly, higher drug potency is associated to the intrapericardial via. For example, in a model of LAD endothelial injury, platelet aggregation reduction was more dramatic when sodium nitroprusside was given intrapericardially than when given intravenously [175]. In turn, only when given intrapericardially (and not subcutaneously) did IGF-1 increase ejection fraction in a model of heart failure [106]. When compared to intracoronary injection, intrapericardial nitroglycerin administration led to an enduring increase of coronary cross-sectional area [173]. In contrast to the intravenous route, intrapericardial delivery of procainamide increased atrial effective refractory period more sustainably. Still, both methods could increase atrial fibrillation thresholds and intravenous injections could prolong atrioventricular and interventricular conduction times. This suggests that while intrapericardial delivery may be suited to modulate atrial electrophysiology, intravenous delivery is indicated to address ventricular electrophysiological perturbances [168]. However, considering previous studies showing a molecular gradient diffusion from the epicardium to the endocardium layers and from the atria to ventricles [7, 170], it is likely that by increasing intrapericardial concentration of the drug one would achieve the same therapeutic effects. Intrapericardial application of drugs also seems to be suitable to address disturbances in cardiac chronotropy and inotropy. For instance, sotalol and atenolol mitigated more efficiently the nitroprusside-induced increase in heart rate and the dobutamine-induced increase in d*P*/d*T* max by the intrapericardial route than by systemic administration in rats [18]. In turn, metoprolol decreased heart rate after tachycardia induction only if directly administrated to the pericardial sac [133]. The minimisation of systemic side effects is yet another great advantage of introducing drugs directly into the pericardial cavity. Blood pressure depressing and reduced peripheral resistance are common secondary effects of therapies involving nitric oxide donor drugs. In this regard, intravenously administered sodium nitroprusside reduced mean aortic pressure and peripheral resistance more intensely [175]. Unlike intrapericardial, intracoronary injection of nitroglycerin transiently increased heart rate and increased blood pressure [173]. Besides, intravenous administration of nitroglycerin decreased blood pressure and increased d*P*/d*T* more strongly than intrapericardial delivery [84]. A big concern with anti-arrhythmic drugs is the narrow therapeutic window. Indeed, a more precise therapeutic control is possible by the intrapericardial channel. For instance, metoprolol given intrapericardially to treat tachycardia and atrial fibrillation did not reduce d*P*/d*T* nor blood pressure, as opposed to metoprolol given intravenously [133]. As the last note, intrapericardial drug delivery is best indicated for acute interventions than for chronic treatments, as chronic intrapericardial catheterisation was associated to moderate inflammation and fibrosis in adjacent areas to the catheter, 6 months after the beginning of digoxin and procainamide therapy [80]. Otherwise, this method is rather safe [84, 148, 168].

Intrapericardial gene therapy

The feasibility, safety, sustainability and efficacy of intrapericardial drug delivery have also motivated researchers to attempt this route for more cutting-edge therapies such as myocardial gene transfer. Indeed, when compared to direct intramuscular and intracoronary injection of β-galactosidase-encoding viruses, intrapericardial injection resulted in a widespread and consistent expression across the surface myocardial layers, without signs of inflammation or toxicity [6]. As observed for drugs introduced in the pericardial sac, a pattern of viral gene expression increasing from myocardium to epicardium and pericardium is observed in this kind of therapy. Higher expression in visceral and parietal pericardium was reported [90, 94, 101], but appreciable expression was also detected in epicardium to mid-myocardium

[90, 94, 101, 183]. The intrapericardial viral-mediated gene therapy was also attempted in pathological contexts, but not always successfully. For instance, a $VEGF_{165}$-encoding adenovirus could not ameliorate myocardial perfusion and fibrosis in a swine model of chronic myocardial ischaemia [94]. Contrarily, HGF-encoding adenovirus improved left ventricle ejection fraction and capillary density in a murine model of myocardial infarction [172]. Nevertheless, intrapericardial delivery of adenoviruses is not void of risks. These treatments may induce pericardial effusions in dogs [94] and higher doses can be lethal in mice [183]. Therefore, despite the potential of viral-mediated gene transfer as a sustained protein release system in the heart, its disadvantages greatly limit clinical translation. Still, minding the remaining studies in Table 3, it should be emphasised that the 'viral factor' is likely the main cause of unsuccess. Hence, the intrapericardial delivery method for drugs or bioactive compounds should not be discouraged.

Intrapericardial stem cell therapy

Stem cell delivery has been tested in animal models of acute myocardial infarction and chronic myocardial ischaemia. Commonly used routes comprise direct intramyocardial injection, epicardial delivery, intracoronary infusion and intravenous administration. However, the former two are invasive, the intracoronary infusion is associated with quick loss of cells due to coronary blood flow or with the coronary blockade, and the latter is related with a very large peripheric distribution of stem cells [164]. Therefore, intrapericardial delivery emerges as a potential alternative addressing the limitations above. Furthermore, the slow pericardial fluid turnover rate may help to prolong stem cell retention, not least because it can store released paracrine factors and perpetuate their therapeutic effects. This has been the rationale followed by Miglino et al. [19] and more recently by Casado et al. [15, 16] to test the efficacy of intrapericardially injected stem cells in swine models of myocardial infarction. The former reported pronounced myocardial homing of bone marrow mononuclear cells at infarcted areas [19]. This finding was then corroborated by Casado's group, showing that stem cells reached infarcted areas of the left ventricle, but also right ventricle and atrium up to 7 days post-injection [16]. Later, they delivered cardiosphere-derived cells in the same animal model. Despite the fact that stem cell administration did not change cardiac function parameters, its therapeutic potential was confirmed by observed immunomodulatory properties ($CD8^{+}CD16^{+}$ effector T cells were decreased 1 month later) and by the decrease of pericardial fluid γ-glutamyl transferase (associated to heart failure severity) and interferon α (inflammation marker) levels [15]. In both cases, the technique was safe and neither cardiotoxicity nor adverse side effects were reported [15, 16]. Prolongation of stem cell therapeutic activity may be achieved using biomaterials such as gelfoam. Indeed, Ladage et al. [86] reported successful engraftment of gelfoam-enable autologous bone marrow-derived mesenchymal stem cells 1 week after delivery in a swine model of myocardial infarction. Besides, these authors reported a new delivery modality using intravascular ultrasound guidance for the safe percutaneous penetration of the pericardial sac. This method offers great clinical translatability. Consequently, as opposed to gene therapy, these first studies in the regenerative medicine turn the intrapericardial route a promising avenue to treat myocardial infarction in humans.

Intrapericardial drug delivery in humans

The ultimate proof of intrapericardial drug delivery safety and efficacy is provided by studies in the human setting (Table 4). Currently the intrapericardial via is widely used to treat pericardial diseases, mainly malignant, purulent and infective pericarditis, in addition to haemopericardium (requiring fibrinolysis) [145]. Concerning malignant pericarditis, the high risk of recurrent pericardial effusions, increasing the risk of cardiac tamponade, after pericardiocentesis has motivated the exploration of the intrapericardial delivery method [13, 14, 42, 63, 92, 102, 142, 161–163]. In patients with malignant pericardial effusion or even cardiac tamponade, administration of cytostatic drugs, such as thiotepa and cisplatin, sclerosing agents, such as minocycline and tetracycline, or radiocolloids, such as ^{32}P-colloid, among others has shown high efficacy (81–100%, mean 96%) in preventing new episodes of pericardial effusion [13, 14, 23, 26, 42, 53, 63, 92, 102, 103, 116, 130, 131, 142, 161–163]. Furthermore, globally these were safe, with only three studies reporting ECG changes, non-self-managed atrial fibrillation, severe chest pain and vasovagal reaction [92, 102, 163]. Less attention has been devoted to other pericardial diseases. For instance, intrapericardial instillation of streptokinase was found to be safe and effective to treat purulent pericarditis [68, 169, 182] In turn, triamcinolone was efficient in treating chronic autoreactive pericarditis, but was associated with iatrogenic Cushing syndrome [100]. The vast majority of intrapericardial therapeutics found in the literature targets pericardial disease. However, with the support of substantial basic research, some strategies have been reported where intrapericardial delivery is used to treat heart disease directly. Beyond some case reports [67, 117, 125, 174], percutaneous intrapericardial fibrin-glue injection therapy was used to treat left ventricle wall rupture in 9 patients with relative success [158]. In turn, BioGlue® (bovine serum albumin and glutaraldehyde) was used to treat cardiac rupture, but with low success [135]. Besides, although not directly targeted to the pericardial cavity, the use of epicardially delivered amiodarone-releasing adhesive

Table 4 The pericardial fluid as a vehicle for drug instillation in humans[a]

Drug	Condition	Patients characteristics	Safety/efficacy	Refs.
Radiolabeled tumour-associated HMFG2[b] antibody	Malignant effusion	Patients with adenocarcinoma of unknown origin ($n=1$), squamous lung carcinoma ($n=1$) and lung cancer ($n=1$)	Safe 100% of the patients ($n=3$) responded positively (complete remission)	[131]
Tetracycline	Malignant effusion	Patients with lung ($n=27$), breast ($n=16$), stomach ($n=3$) and ovary cancer ($n=1$), adenocarcinoma of unknown origin ($n=7$), mesothelioma ($n=2$), leukaemia ($n=1$) and lymphoma ($n=1$)	Safe (self-resolved complications included transient arrhythmias, $n=5$, fever, $n=5$, and pain, $n=9$) 81% of the patients ($n=43$) responded positively: 74% ($n=43$) had effusions controlled > 30 days[c] and 9% ($n=5$) died due to disease progression, but without evidence of effusions 14% of the patients ($n=8$) displayed uncontrolled effusions[d]	[142]
Cisplatin	Malignant effusion	Patients with Hodgkin's disease ($n=2$), breast cancer ($n=2$), malignant thymoma ($n=1$) and pleural mesothelioma ($n=1$)	Safe (mild nausea) 100% of the patients ($n=6$) responded positively: 50% ($n=3$) showed a complete remission and 50% ($n=3$) died due to disease progression, but without evidence of effusions	[42]
OK-432[e]	Malignant effusion	Patients with breast ($n=3$), lung cancer ($n=3$), adenocarcinoma of unknown origin ($n=2$), multiple myeloma ($n=1$) and hepatocellular carcinoma ($n=1$)	Safe (manageable fever, chest pain and hypotension, transient arrhythmias and PVC) 100% of the patients ($n=10$) responded positively. None showed recurrent effusions. 8 patients died due to disease progression	[63]
Oxytetracycline	Malignant effusion	Patients with breast ($n=7$), stomach ($n=2$) and lung cancer ($n=2$)	Safe (mild pain, transient fever, PVC, catheter infection, requiring replacement) 100% of the patients ($n=11$) responded positively. None showed recurrent effusions. All patients died due to disease progression	[53]
Minocycline	Malignant effusion	Patients with lung ($n=5$), breast ($n=3$), oesophagus ($n=1$), stomach ($n=1$) and ovary cancer ($n=1$), lymphoma ($n=1$), desmoid tumour of the chest ($n=1$) and pericardial mesothelioma ($n=1$)	Relatively safe (severe chest pain, ECG changes suggestion pericardial or subepicardial injury, transient fever, vasovagal reaction) 71% of the patients ($n=10$) responded positively 29% of the patients ($n=4$) had recurrent effusions 10 patients died due to disseminated carcinoma	[92]
Cisplatin	Malignant effusion	Patients with lung cancer ($n=16$)	Safe (mild nausea and transient atrial fibrillation) 94% of the patients ($n=15$) responded positively 6% of the patients ($n=1$) did not respond, after 4 courses of treatment All patients died due to disease progression	[162]
Cisplatin	Malignant effusion	Patients with lung ($n=3$), colon ($n=1$) and ovary adenocarcinoma ($n=1$), pericardial mesothelioma ($n=1$), angiosarcoma ($n=1$), small cell ($n=1$) and squamous cell lung cancer ($n=1$)	Safe (mild nausea, transient arrhythmia, bacterial pericarditis) 100% of the patients ($n=9$) responded positively. One patient died due to pulmonary embolism All deaths were due to disease progression	[161]
Minocycline	Malignant cardiac tamponade	Patients with lung ($n=3$), breast ($n=2$), bladder ($n=1$) and colon cancer ($n=1$), mesothelioma ($n=1$) and leukaemia ($n=1$)	Relatively safe (irritant, occasional severe pain, but without ECG changes) 100% of the patients ($n=9$) responded positively. 6 patients died with cardiac tamponade-unrelated causes	[102]

Table 4 (continued)

Drug	Condition	Patients characteristics	Safety/efficacy	Refs.
Thiotepa[f]	Malignant effusion	Patients with breast ($n=9$) and lung cancer ($n=11$), unknown primary tumour ($n=1$) and metastatic melanoma ($n=1$)	Safe (transient thrombocytopenia and/or leukopenia in 2 patients) 83% of the patients ($n=19$) responded positively[c] 17% of the patients ($n=4$) did not respond 17 patients died of disease progression, of which 7 also displayed progressive pericardial effusion	[23]
Streptokinase	Purulent pericarditis	Patients (children) were infected with *Staphylococcus aureus* ($n=4$). Unidentified microorganisms in two patients	Safe (fever, self-resolved or manageable right atrial masses) 83% of the patients ($n=5$) showed complete pus clearance 17% of the patients ($n=1$) elicited intrapericardial haemorrhage with submitral pseudoaneurysm No pericardial constriction detected in the follow-up (13–30 months)	[68]
^{32}P-colloid	Malignant effusion	Patients with breast ($n=23$) and lung cancer ($n=8$), lymphomas and gastrointestinal tumours (n = 5)	Safe (transient tachycardia) 95% of the patients ($n=34$) responded positively 5% of the patients ($n=2$) did not respond All patients died due to disease progression	[26]
Cisplatin	Malignant effusion	Patients with breast ($n=2$) lung ($n=1$) and ovary cancer ($n=1$), mediastinal fibrosarcoma ($n=1$) and unknown primary tumour ($n=1$)	Safe 100% of the patients ($n=6$) responded positively None showed recurrent effusions	[130]
Thiotepa[f]	Malignant cardiac tamponade	Patients with breast cancer ($n=19$)	Safe 100% of the patients ($n=19$) responded positively. Small recurrent effusions were found in 4 patients (after 2 and before 6 months of follow-up) All deaths were due to disease progression	[14]
Carboplatin	Malignant effusion	Patients with non-small cell lung cancer ($n=10$)	Safe 90% of the patients ($n=9$) responded positively: 80% ($n=8$) had effusions controlled > 4 weeks and 10% ($n=1$) required drainage after 4 weeks 10% of the patients ($n=1$) did not respond All patients died due to disease progression	[116]
Triamcinolone	Chronic autoreactive pericarditis	Patients with pericarditis or myocarditis ($n=84$)	Relatively safe (iatrogenic Cushing syndrome) 90% of the patients ($n=76$) responded positively, showing no signs of pericardial effusion after 3 months 10% of the patients ($n=8$) did not respond to either of the doses tested Lower dose was as effective, but with lower side effects	[100]
Cisplatin	Malignant effusion	Patients with non-small ($n=36$) or small cell lung cancer ($n=3$), pleura ($n=1$) and pericardial mesothelioma ($n=1$), lung angiosarcoma ($n=1$), uterine ($n=1$) and colon adenocarcinoma ($n=1$), malignant melanoma ($n=1$), ovary cancer ($n=1$)	Relatively safe (atrial fibrillation, sclerosis without pericardial constriction) 94% of the patients ($n=43$) responded positively 6% of the patients ($n=3$) did not respond All patients died due to disease progression	[163]

Table 4 (continued)

Drug	Condition	Patients characteristics	Safety/efficacy	Refs.
Thiotepa	Malignant effusion	Patients with non-small cell lung cancer ($n=16$), breast ($n=11$) and endometrial cancer ($n=1$), microcytoma ($n=1$) and melanoma ($n=1$)	Safe 100% of the patients ($n=33$) responded positively[c] All deaths were due to disease progression	[103]
Streptokinase	Purulent pericarditis	Patients were infected with *Streptococcus species* ($n=1$), *Streptococcus intermedius* and *Enterococcus faecalis* ($n=1$). Unidentified microorganisms in one patient.	Safe 100% of the patients ($n=3$) showed complete pus clearance No pericardial constriction detected in the follow-up (2–9 months)	[182]
Cisplatin	Malignant cardiac tamponade	Patients with lung adenocarcinoma ($n=24$)	Safe (transient atrial fibrillation, non-sustained ventricular tachycardia) 92% of the patients ($n=22$) responded positively 8% of the patients ($n=2$) did not respond All deaths were due to disease progression	[13]
Fibrin (glue)[g]	Left ventricle free wall rupture	Patients with left ventricle wall rupture secondary to acute myocardial infarction ($n=9$)	Relatively safe (one patient had uncontrolled bleeding after pericardiocentesis) 67% of the patients ($n=6$) responded positively, being free of cardiovascular events during follow-up (> 3 years). One patient died due to noncardiac causes 33% of the patients ($n=3$) did not respond: one had uncontrolled bleeding requiring surgical intervention and two suffered from rerupture before 1 week	[158]
Streptokinase	Purulent pericarditis	Patients were infected with *Staphylococcus aureus* ($n=8$). Unidentified microorganism in one patient	Safe 100% of the patients ($n=9$) showed complete pus clearance No pericardial constriction detected in the follow-up (6 months)	[169]
BioGlue® (bovine serum albumin + glutaraldehyde	Cardiac rupture	Patients with cardiac rupture secondary to pacemaker implantation or to acute myocardial infarction ($n=19$)	Safe (residual bleeding, non-sustained ventricular tachycardia) 26% of the patients ($n=5$) responded positively, showing no pericardial constriction nor ventricular pseudoaneurysm, 1 year after 74% of the patients ($n=14$) did not respond: 12 died immediately, one died at 1 month due to multi-organ failure and the other died after 1 year due to myocardial infarction	[135]

ECG electrocardiogram, *PVC* premature ventricular contractions

[a]Case reports with less than 3 subjects were not considered

[b]Mucin-1 (tumour-associated antigen)

[c]Smith's criteria: 30 days cutoff to assess the outcome of the treatment of pericardial effusions. Additional criteria are the absence of symptoms and the lack of the need to repeat pericardiocentesis

[d]Remaining 2 patients (3%) could not receive tetracycline intrapericardially due to technical hurdles: catheter could not be inserted or there was unresolved intracatheter clotting

[e]Penicillin-treated and heat-treated lyophilised Streptococcus pyogenes A3

[f]Triethylenethiophosphoramide

[g]Fibrin glue contained fibrinogen, coagulation factor XIII, aprotinin and thrombin

hydrogels was shown to reduce postoperative atrial fibrillation [39]. Thus, percutaneous intrapericardial administration of amiodarone and other drugs already delivered onto the epicardial surface should be tested. Ultimately, these may provide the means for a less invasive and safer route for drug administration.

What can we expect from pericardial fluid research in the decade to come?

The diagnostic and prognostic potential of pericardial fluid and the efficacy of the intrapericardial route for drug administration has now been established. Nevertheless, a long road is yet to be explored, before the full potential of pericardial fluid can be determined. Four main areas of research are identified that are beginning to sprout and that are foreseen to bring breakthroughs in pericardial fluid research in the next decade.

Profiling of the pericardial fluid miRNome library

The application of high-throughput technologies is expected to accelerate the identification of miRs with diagnostic, prognostic and therapeutic properties. Following a targeted approach, Miyamoto et al. [112] quantified the pericardial fluid levels of miR-423-5p, miR-133a, miR-126 and miR-92a in subjects with stable or unstable angina as well as with aortic stenosis, although no differences were discovered. Still, the helpfulness of miRNomics has already been witnessed. For instance, Beltrami et al. [10] demonstrated that exosomes collected from the human pericardial fluid improve angiogenesis and post-ischaemic blood flow recovery in a mouse model of unilateral limb ischaemia. In the same study, the miRNA let-7b-5p was identified as a key pro-angiogenic player, after performing a miRNA array. These encouraging results anticipate that the application of high-throughput techniques, such as "sequencing-by-synthesis", will help uncover heart-specific miRNAs with prognostic and therapeutic value. Besides the possibility of identifying new miRNAs with this approach, it confers an additional advantage over standard qPCR and microarray analysis [149].

Characterisation of the pericardial fluid proteome

By collecting and analysing samples in different pathophysiological backgrounds, the complete spectrum of proteins will be uncovered, new biomarkers and therapeutic targets will be added to the cardiology setting. Hitherto, the proteomic characterisation of pericardial fluid exosomes (collected from patients that have suffered myocardial infarction) has identified clusterin as an important adjuvant for myocardial recovery after an ischaemic insult [43]. Furthermore, the biomarker potential of different proteins/peptides panels (as represented in Figs. 2 and 3) shall be determined by new MS-based approaches such as multiple reaction monitoring. This technique confers several advantages over the currently applied immunoassays, namely the higher sensitivity and specificity, the excuse of the expensive high-quality antibodies, the chance to scrutinise multiple protein isoforms and post-translational modifications and, perhaps more importantly, the possibility to provide in much less time a multiplex analysis of over a hundred proteins, in a single run [50].

Optimisation of intrapericardial drug delivery

Advancements in drug delivery systems will maximise the therapeutic benefits of intrapericardial drug instillation. For instance, poly(lactic-*co*-glycolic acid) nanoparticles loaded with BODIPY (a fluorophore) administered in a single dose to the pericardial cavity or rabbits resulted in a cargo half-life of a week. Besides, the fluorophore could penetrate the myocardium following a transmural gradient, while the nanoparticles were confined to epicardial layers and the pericardial cavity [138]. Therefore, nanoparticles-induced sustained release of bioactive compounds may prove to be efficient in increasing pericardial residence time and achieving long-term therapeutic benefits. Besides, improved safety of percutaneous approaches to drug delivery (e.g. assisted by echocardiography [103, 116, 142]) will open the path for new cardiac treatment strategies confined to the pericardial sac.

Cardiac regenerative medicine

Pericardial fluid's low turnover rate has drawn attention to researchers working in this field. Direct intrapericardial delivery of stem cells is emerging and the number of studies is expected to rise. Moreover, system approaches aiming at the characterisation of the miRNome, proteome and metabolome of pericardial fluid in different pathological settings may elicit new therapeutic factors. These may be used as part of the 'paracrine paradigm' currently set on regenerative medicine and can themselves be intrapericardially delivered to stimulate endogenous cardiac repair mechanisms.

Conclusions

If previously considered a bare plasma ultrafiltrate without readily palpable applications, today's perspective on pericardial fluid has changed radically. Accumulating evidence shows that pericardial fluid is a stable biofluid with low clearance rates and stocking heart-derived factors released from regions as deep as the myocardium. Hence, the exploration of its diagnostic, prognostic and therapeutic properties has gained popularity. However, the relative difficulty in the collection and the ethical limitations of using samples from healthy individuals has lagged the knowledge of pericardial fluid features over other biofluids commonly used in clinical practice and in research, such as serum/plasma and urine. This explains why the behaviour of pericardial fluid and its molecular profile is better known for pericardial diseases, as these often require urgent pericardial drainage. It is pretty much settled that pericardial fluid analysis is important to discern the aetiology of pericarditis (e.g. total protein, LDH; PCR for tuberculous pericarditis, but also ADA, lysozyme and IFNG; tumour markers such as CEA for suspected neoplasms and triglycerides for chylopericardium) as recently recommended by ESC guidelines on the diagnosis and management of pericardial diseases [2]. Nonetheless, the perioperative analysis of pericardial fluid (easily and safely accessed during surgery) may be of great value in the future to predict the outcome of the patients undergoing cardiac surgery, to design risk stratification algorithms and, ultimately, to tailor personalised adjuvant pharmacotherapies. Indeed, our systematic review elicited several potential markers deserving further research to validate their prognostic potential. These include endostatin and angiostatin to predict coronary collateralisation and FGF1 and VEGF to predict angina stratification in patients undergoing coronary revascularisation (CABG); insulin-like growth factor-1 and matrix metalloproteinase 2 to predict left ventricular reverse remodelling on occasion of aortic valve replacement; perioperative MB to anticipate emergent or recurrent episodes of myocardial infarction (useful for preventive purposes) and, finally asymmetric dimethylarginine, endothelin-1 and mature ADM to anticipate or assess the severity of heart failure.

Beyond the clinical applicability, the close contact between pericardial fluid and the heart makes this biofluid a great vehicle for mechanistic studies. Reported effects of pericardial fluid elements are sundry and comprise regulation of coronary blood flow, modulation of cardiac electrophysiological properties as well as tuning of heart chronotropy and inotropy. Notably, the pericardial fluid has demonstrated potent paracrine activity with important therapeutic implications ex vivo. In the opposite direction, intrapericardial delivery of bioactive compounds, drugs, genes and stem cells is showing itself a promising avenue to bypass the limitations of more traditional routes such as intravenous, intracoronary or intramyocardial ones. Intrapericardial therapeutics is associated with higher heart deposition/retention, higher residence time, lower clearance rates, higher potency/efficacy and lower, if any, systemic side effects. Albeit the limitations of intrapericardial delivery, namely the invasiveness (as compared to intravenous injections) and the transmural gradient attained for the agent, recent advances in drug delivery methods are expected to circumvent these. Particularly, percutaneous echo- or fluorescence-guided pericardial sac perforation and delivery of agents in sustained-release formulas, such as nanoparticles, gelfoams and other biomaterials, will assist in the clinical translation process.

Thus, continuous collection and screening of pericardial fluid from patients undergoing heart surgery and investment in intrapericardial therapy are strongly needed. These approaches will certainly speed the pace towards personalised medicine by prompting effective prognostic tools as well as by pushing towards effective, targeted and non-toxic heart therapy.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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