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Review article

# Edema and fluid dynamics in connective tissue remodelling

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ABSTRACT

The review describes the role of loose connective tissues with focus on transcapillary exchange and edema formation with relevance for inflammation, fibrosis and tumors. Based on studies in these tissues, comparisons are made to the fibrotic processes in the heart.



**Contents**



## 1. Introduction

Remodelling of the cardiac connective tissue following an injury or perturbation is dependent on first, the stimuli triggering the change and second, a cellular response and a remodelling that also will involve local cytokines and growth factors. The primary insult triggering the remodelling can be a myocardial infarction with loss of cardiac muscle tissue, which with time is replaced by a fibrotic scar tissue. However, fibrosis may also result following hypertension [1], edema [2] and other disease processes such as chronic inflammatory lesions in the heart.

We will base this review on observations in particular from studies of the loose connective tissues in skin and carcinomas performed over the recent years in our laboratories and make relevant extrapolations and comparisons to the heart. Our studies of the connective tissue have had a starting point from studies on transcapillary exchange and on the involvement of the extracellular matrix (ECM) in this exchange. We have demonstrated that loose connective tissues that embed peripheral blood vessels can alter interstitial fluid pressure ($P_{IF}$) via an active involvement of connective tissue cells. This cellular control is

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mediated through tension exerted on the extracellular fibre network by the connective tissue cells using collagen-binding β1 integrins. The loose connective tissue contains several fibre networks [3]. These networks compress an expanding ground substance of hyaluronan and glycosaminoglycans [4]. When this compression is altered, so is $P_{IF}$, in turn acting to influence the transcapillary transport and interstitial fluid volume.

Two issues will be dealt with in some detail regarding the heart and fibrosis. First, is the observation that in the heart excess interstitial fluid, *i.e.* edema, will cause interstitial fibrosis in the heart resulting in impaired heart function [2,5,6]. This is different from the skin, lung and several other organs where edema may develop and disappear without harming the organ or leaving any trace of damage, whereas long term edema *e.g.* in conjunction with chronic inflammation results in fibrosis [2,5,6]. Second, is the observation that by altering the integrin expression, notably the β3-integrin, a different phenotype appears with an altered permeability in the cardiac capillaries and evidence for cardiac inflammation and diminished cardiac function [7,8].

## 2. The interstitial matrix and transcapillary exchange

The transcapillary fluid flux is controlled by the hydrostatic and colloid osmotic pressures in the capillaries and in the interstitial fluid. The net transcapillary filtration pressure ($\Delta P$) is the imbalance in these pressures. It is around 0.5 to 1 mm Hg in skin and skeletal muscle in a normal situation [9] and is the driving force for the transcapillary fluid flux, Jv, where $Jv = \Delta P^{*}CFC$ where CFC is the transcapillary filtration coefficient or "water permeability" of the capillary. Furthermore:

$$Jv = \Delta P^{*}CFC = CFC(P_C - P_{IF} - \sigma(COP_C - COP_{IF}))$$

where *P* and COP are hydrostatic and colloid osmotic pressures across the capillary, respectively, while subscripts C and IF denote capillary and interstitial fluid, respectively. Finally, $\sigma$ is the osmotic reflection coefficient of plasma proteins across the capillaries.

In a normal steady state the net transcapillary fluid flux equals lymph flow to maintain a constant interstitial volume [9]. Normally, alterations in transcapillary flux are "autoregulated" by adjustments in the transcapillary pressures that will limit further changes in the flux: an increased fluid flux caused by increasing capillary hydrostatic pressure or lowering plasma colloid osmotic pressure, will be counteracted by an increase in $P_{IF}$ and a fall in interstitial colloid osmotic pressure. Reabsorption of fluid from the interstitial space into the capillary will likewise cause a lowering of $P_{IF}$ and an increase in interstitial colloid osmotic pressure that will also resist further changes in interstitial volume. Thus, the interstitial volume is normally well controlled or "autoregulated" within narrow limits by a readjustment of the interstitial pressures in response to the altered capillary filtration [9]. If such an autoregulation is not sufficient to counteract the changes across the capillary wall, edema, *i.e.* visible swelling, will result. It should be noted that the localized edema may occur by redistribution of the extracellular fluid volume to one organ or within an organ. However, generalized edema as seen *e.g.* in nephrotic syndrome or heart failure requires retention of fluid and salt and involves an expansion of the extracellular volume [9].

## 3. Acute inflammation and edema formation

The interstitial fluid normally requires 24–48 h to turn over in skin or skeletal muscle. Since edema associated with inflammatory reactions can occur in a few minutes, this attests to an increase in transcapillary fluid flux which is several hundred times above the normal filtration rate. As part of the inflammatory response there is an increase in capillary filtration coefficient (capillary permeability to water) by 2–3 times the normal value [10,11]. Due to the small increase in capillary filtration coefficient, there must be a correspondingly large increase in the net capillary filtration pressure, and as will be elaborated in detail below, the major change to create this rapid filtration results from a lowering of $P_{IF}$ during the first minutes and up to 1 h after the onset of inflammation (see below). However, as edema is formed, $P_{IF}$ will increase and during a steady state when the edema has been established, it is the increased permeability together with an increased capillary hydrostatic pressure that will maintain the edema once it has formed. In this situation, the $P_{IF}$ will return to its normal function as part of the edema preventing mechanisms where an increase in interstitial volume will raise $P_{IF}$ which in turn acts across the capillary wall to limit further fluid filtration.

## 4. Loose connective tissue and its role in edema formation

Loose connective tissue elements are present in all organs outside the central nervous system. They embed blood vessels and underlie mucosal surfaces. The ECM of the loose connective tissue constituting the interstitial matrix has three principal components: 1) collagens constituting the stiff scaffolding for organs and organisms; 2) elastic fibers and microfibrils; and 3) the ground substance composed from proteoglycans and hyaluronan, as well as glycoproteins. This interstitial matrix provides the route of transport for nutrients and waste materials between the abluminal side of the endothelial barrier to the parenchymal cells of any tissue. The composition of the interstitial matrix, *i.e.* the amount and type of the fibrous scaffolding and ground substance, in concert with connective tissue cells determine the physical properties for convective and diffusive movement of molecules in the tissue.

The loose connective tissue surrounding blood vessels was commonly thought of as a "passive" framework in the sense that its physical properties such as diffusivity, hydraulic conductivity, compliance and $P_{IF}$ remain fairly constant except in conditions such as fibrosis. This concept of a static and passive loose connective tissue has been challenged based on two lines of research. First are the observations that both synthesis and degradation of the matrix components are rapid processes taking place locally in the tissue and are influenced by many factors including the fluid flux through the tissue [12–14]. Second are the unexpected observations that, during acute inflammation, $P_{IF}$ in the tissues falls, rather than increases, within minutes and for up to 1 h after the inflammatory challenge. During this time edema develops. This implies the existence of a $P_{IF}$ that generates rather than opposes edema formation associated with the inflammation. Thus, rather than preventing the edema formation in this situation, $P_{IF}$ will "actively" promote it [15]. In fact, following a second degree burn injury $P_{IF}$ fell as low as −150 mm Hg, providing the "active" driving force required for the rapid edema formation [16]. The rapid lowering of $P_{IF}$ demonstrates that the loose connective tissues can alter their physical properties within minutes, thereby influencing the balance of forces controlling transcapillary water and solute movement.

Since the initial observations, a combination of *in vivo* studies and *in vitro* studies using fibroblasts in collagen gels as a model system have enabled us to propose a mechanism of contraction as summarized in Fig. 1. The basis for the lowering of $P_{IF}$ is that the interstitial matrix swells when given free access to fluid due to its polyanionic and therefore osmotically active, glycosaminoglycans, in particular hyaluronan. The swelling is balanced *in vivo* by constraining microfibril and collagen networks [3,17]. The dynamic balance between relaxation and contraction of the interstitium by the connective tissue cells influence $P_{IF}$ and thereby transcapillary fluid flux. In rat skin this phenomenon is mediated by the laminin and collagen-binding integrin $\alpha_2\beta_1$ [18]. The integrins are transmembrane heterodimeric cell surface receptors mediating cell–cell and cell–matrix adhesion built from of an α- and a β-unit and capable of inside–out and outside–in signalling [19]. As indicated in our model in Fig. 1, tissue hydration, permeability and $P_{IF}$ reflect the balance

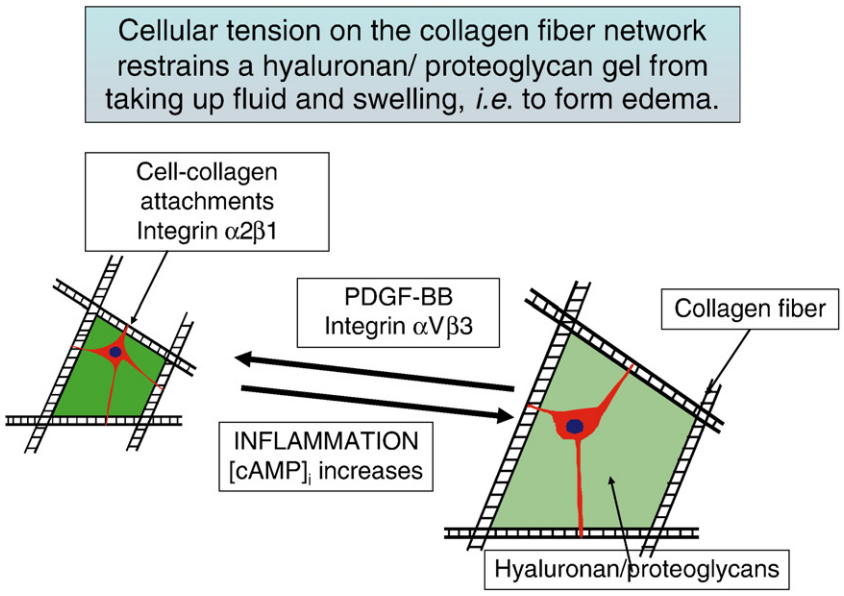


**Fig. 1.** Proposed mechanism for dynamic control of interstitial fluid pressure ($P_{IF}$). Dextran anaphylaxis, prostaglandin E1 and antibodies toward $\alpha_2\beta_1$-integrin loosens the cell attachment on the collagen fibers and the tissue swells due to the content of hyaluronan and glycosaminoglycans. The decreased compaction lowers $P_{IF}$ and causes fluid influx. Platelet-derived growth factor BB (PDGF-BB), insulin and prostaglandin F2α causes connective tissue cells to compact the collagen fibers resulting in restoration of the normal compaction and normal $P_{IF}$ of the tissue. See text for further explanation.

between the osmotic, or swelling pressure, generated by the counterions of the glycosaminoglycans, particularly hyaluronan in the ground substance and tension generated on the fibrous proteins by the connective tissue cells.

Our proposed mechanistic model holds that connective tissue cells apply tensile forces on ECM fibers that in turn restrain the underhydrated ground substance from taking up fluid and swell. A decrease in cellular tension on the ECM fibers allows the ground substance to swelling, *i.e.* form edema. During this process negative $P_{IF}$ values can be recorded if refilling of the tissue with fluid is inhibited. The tensile forces are mediated by $\beta_1$-integrins, in rat dermis the collagen-binding integrin $\alpha_2\beta_1$ is of particular importance [20]. Furthermore, they depend on the cytoskeleton and can be pharmacologically modulated [18,21–23]. Dermal $P_{IF}$ lowered after anaphylaxis can be normalized by instilments of platelet-derived growth factor (PDGF) BB or insulin [20,24,25] by an integrin $\alpha_V\beta_3$-dependent mechanism [26,27]. Our data suggest that whereas $\beta_1$-integrins participates in fluid homeostasis, $\beta_3$-integrins participate in $P_{IF}$-recovery after inflammation-induced lowering of dermal $P_{IF}$.

Fibroblast-mediated collagen-gel contraction is stimulated by PDGF-BB and inhibited by pro-inflammatory agents such as prostaglandin $E_1$ (PGE1) and interleukin (IL)-1 [21,28]. Recently, we and others have identified at least two mechanisms for cell-mediated collagen-gel contraction [26,29]. The collagen-binding integrins, $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ all mediate rapid contraction that proceeds in serum-free media. Contraction mediated by the integrin $\alpha_2\beta_1$ requires integrin-elicited signaling [29]. When the collagen-binding $\beta_1$ integrins either are absent or their signaling and/or activity perturbed a second mechanism can become operative. This mechanism requires stimulation by PDGF-BB or insulin and depends on the RGD-dependent integrin $\alpha_V\beta_3$ [26,27,29]. Our data suggest that gel contraction *in vitro* can serve as an *in vitro* model for control of $P_{IF}$ *in vivo*.

## 5. Conditions and diseases altering interstitial fluid pressure

Lowering of $P_{IF}$ has been demonstrated in several inflammatory reactions and conditions with tissue injury such as burn injury, frost bite injury which both involve a physical damage to the tissue [30]. Furthermore, a lowering of $P_{IF}$ also takes place in anaphylaxis, complement activation, acute asthma and neurogenic inflammation [30]. Also, it can be induced by PGE1 and PGI2, endotoxin, septicaemia, IL-1 and -6, tumor necrosis factor (TNF) [31], carrageenan and the cytoskeletal acting agents cytochalacin D and taxanes [32] as well as platelet activating factor [33].

Conversely, other prostaglandins and cytokines can reverse the lowering of $P_{IF}$ as can prostaglandin F2α, corticotropin releasing factor, mystixins and α-trinositol (D-myo-1,2,6-inositol-trisphosphate) and vitamin C [30]. Thus, the loose connective tissues seem to operate around a set-point where some endogenous substances can lower $P_{IF}$ while others can raise it. The pressure excursions in acute inflammation are in the order of up to 10 mm Hg, and this is substantial compared to a normal net filtration pressure across the capillary wall of 0.5–1 mm Hg. The lowering of $P_{IF}$ *in vivo* can therefore increase the capillary filtration pressure by 10 to 20 times its normal value.

Many aspects of the mechanisms of integrin action in lowering $P_{IF}$ are still unclear, but our current understanding is that a series of mediators induce reactions that alter the functions of the integrin system. Studies of fibroblast-mediated collagen-gel contraction have been important for screening substances that potentially have an effect on the integrin–collagen interaction and thereby assembly and biophysical properties of the extracellular matrix. In this assay, water soluble collagen I is mixed with fibroblasts and the rate of collagen compaction is monitored. Using this assay, we have screened a large number of substances for their ability to increase or decrease the compaction rate and based on such experiments moved on to test the *in vivo* effects of these same substances. In short, we have found a parallel behaviour between the *in vivo* and *in vitro* experiments where substances that decrease the rate of collagen-gel compaction will lower $P_{IF}$ while substances that increase the rate of compaction will increase $P_{IF}$. Using a combination of the *in vivo* and *in vitro* techniques, we have demonstrated that under normal conditions the collagen-binding integrin $\alpha2\beta1$ is of particular importance for the control of $P_{IF}$ in rat dermis and plays a crucial role in the lowering of $P_{IF}$ [18,34].

## 6. Reversal of $P_{IF}$

As a continuation of the studies of $P_{IF}$, we have also been able to demonstrate that PDGF-BB is able to reverse a lowered $P_{IF}$ in a

dose-dependent manner [18]. Later also several other pharmaceutical agents and cytokines have been demonstrated to reverse a lowered $P_{IF}$. The procedures that have been used to demonstrate the role of the reversal of $P_{IF}$ have been by causing a general inflammation using *e.g.* the mast cell degranulating substance Compound 48/80 (C48/80) intravenously and subsequently injecting the cytokine or agent locally that reverses $P_{IF}$. Based on combined *in vivo* and *in vitro* experiments, an important observation is that the reversal of $P_{IF}$ involves the αVβ3-integrin [26] since in $\beta_3$-integrin null mice, PDGF-BB is not able to return $P_{IF}$ to its normal level when PDGF-BB is administered subsequently to a lowering of $P_{IF}$ induced by C48/80, *i.e.* the growth factor could not normalize $P_{IF}$ in the dermis of such animals. Thus, our working hypothesis is therefore that under normal conditions tension from the cells to the dermal fibres is maintained by $\beta_1$-integrin-mediated contraction. Thus, pro-inflammatory mediators release the $\beta_1$-integrins resulting in lowering of $P_{IF}$ and edema formation. PDGF-BB counteracts the tendencies to edema through stimulating the activity of the $\alpha_V\beta_3$-integrin.

## 7. Intracellular signalling pathways

Intracellular signalling pathways involved in lowering of $P_{IF}$ have been studied using a combination of *in vivo* and *in vitro* methods. The former studies have included use of transgenic mice with modifications in the intracellular signalling pathways between the $\beta_1$–integrin system and PDGF-BB [24].

Using the combination of the collagen-gel contraction assay and *in vivo* animal experiments, we have demonstrated that under normal homeostasis the collagen-binding integrin $\alpha_2\beta_1$ is of particular importance for control of $P_{IF}$ in rat dermis. This integrin also plays a crucial role in the lowering of $P_{IF}$ [18,34]. Further, PDGF-BB can reverse the anaphylaxis induced lowering of $P_{IF}$ [20] and by also using transgenic mice, we demonstrated that this *in vivo* function of PDGF is dependent on PI3'-kinase binding-site at the PDGF-BB receptor since knockout lesion of the PI3'-kinase abolished the ability of PDGF to restore $P_{IF}$ in mice [24].

## 8. Tumors and chronic changes

Carcinomas exhibit infiltrating myeloid cells, distorted blood vessels, hypoxia, low pH and activated connective tissue cells that commonly produce a fibrotic ECM [35–37]. Thus, a carcinoma stroma shares many traits with conditions of chronic inflammation and wound healing. Further, carcinoma stroma is characterized by pathologically high $P_{IF}$. Together, these characteristics lead to a disturbed physiology that impedes the uptake of anti-cancer drugs into the carcinoma [35,36,38–40]. Signals that affect stroma cells are mediated by adhesion receptors such as integrins and by receptors for growth factors and cytokines.

We and others have established a correlation between lowering tumor $P_{IF}$ and uptake and efficacy of chemotherapeutic agents in experimental carcinoma [41–45]. Notably, by using microdialysis or MR, we have *pro forma* demonstrated that lowering of carcinoma $P_{IF}$ increased capillary-to-interstitium transport of low-molecular weight molecules including 5-fluorouracil (5FU) in experimental carcinoma [44,46,47]. Interestingly, treatment with a specific inhibitor of transforming growth factor (TGF)-β1 and -β3, or with a specific inhibitor of carcinoma cell-derived VEGF (Bevacizumab), both agents lower $P_{IF}$ and also reduce leakage of plasma proteins (normalize $\sigma$). This results in a decreased uptake of anti-tumor cell directed antibodies [48] and are in line with the notion of a normalization of tumor blood vessels [36]. Thus, $P_{IF}$ either forms or reflects a barrier for transport of low-molecular drugs into carcinomas, whereas transport of high-molecular weight drugs *e.g.* antibodies are restricted by vascular wall protein leakage (*i.e.* by $\sigma$).

Specific inhibition of TGF-β1 and -β3 reduces MHC class II expression, IL-1β, S100A9 and density of F4/80 positive tumor-associated monocytes/macrophages (TAMs), as well as $P_{IF}$ in xenograft carcinoma [45]. In addition, treatment of animals with a specific inhibitor of IL-1 or with dexamethasone reduces tumor $P_{IF}$. Our data thus point to that TAMs play a role for the distorted physiology in carcinoma stroma. Inflammatory processes regulate $\sigma$ (see above) and we have demonstrated a relationship between carcinoma cell-derived VEGF/VPF, TAMs, plasma protein leakage, extracellular volume and tumor $P_{IF}$ [49]. Installment of PGE1 around carcinomas rapidly and transiently lowers $P_{IF}$ and forms tumor edema [44,46] in line with our previous findings that PGE1 inhibits collagen-gel contraction and lowers $P_{IF}$ in dermis [21]. Thus, it seems reasonable to assume that increased contractility of tumor fibroblasts in part is responsible for the high $P_{IF}$ in carcinoma. Furthermore, we have shown that the small leucine-rich proteoglycan (SLRP) fibromodulin directs the generation of a dense and 'stiff' ECM in carcinoma [50]. Fibromodulin is not expressed in normal loose connective tissues but is expressed in conditions of inflammation and fibrosis. The expression of fibromodulin in carcinoma is down-regulated after treatment of the animals with recombinant IL-1 receptor antagonist or dexamethasone, suggesting that inflammatory processes can regulate fibromodulin expression. In conclusion, $P_{IF}$ is controlled at several levels but with tumor-associated MHC class $II^+F4/80^+$TAMs, as a potential common denominator.

## 9. The heart

There are similar suggestions that there is an important role for the loose connective tissues contributing to the overall organ function also in the heart, although it has so far not been extensively studied. The same ECM components are present in the heart as in the skin although at other concentrations and contents. The content of collagen in the heart is around 20 mg/g dry tissue in rat heart [2] which is somewhat less than in skeletal muscle and about one tenth that of skin [9]. Corresponding figures for the glycosaminoglycan hyaluronan is 0.5 mg/g dry weight in the heart and 2 mg/g dry weight in skin [51]. However, since the interstitial volume in skin is normally about 2–3 times larger than in heart and skeletal muscle, the concentration is still less than in skin. In a fibrotic process, deposition of collagen III seems to be more prominent than deposition of collagen I [2]. In a recent and extensive review, Spinale [52] describes the ECM as a highly dynamic structure "that directly contribute to adverse myocardial remodelling following myocardial infarction, with hypertensive heart disease and with intrinsic myocardial disease such as cardiomyopathy". This extensive review summarizes in detail the important role and involvement of matrix metalloproteinases in myocardial matrix remodelling in the normal situation and in disease states.

In mice deficient in the $\beta_3$-integrin, there is clearly altered cardiac function with a mild cardiac hypertrophy associated with systolic and diastolic dysfunction [8]. Furthermore, the mice had a mild cardiac inflammation that was worsened by transverse aortic constriction. It was concluded that blood-borne cells were at least partially responsible for the hypertrophy and inflammation, suggesting that the expression of $\alpha_V\beta_3$ in bone marrow has a general and suppressive effect on cardiac inflammation.

Furthermore, in mice lacking $\beta_3$-integrins, the development of coronary capillaries fail in male, but not in female mice [53]. These authors observed an enhanced effect of VEGF signalling which contributed to the β3-null phenotype since inhibitors to VEGF and Flk-1 did not normalize the vessels. VEGF injected intravenously into wild type mice induced a similar angiogenic phenotype. Also and in line with altered vascular phenotype are the observations that capillary extravasation and permeability is lowered in the heart of mice lacking $\beta_3$-integrin, while it is not affected in other organs [7]

**Table 1**
Suggested future studies.

| |
|---|
| Collagen-gel contraction studies using cardiac fibroblasts from: |
| Normal hearts |
| Hearts with fibrotic lesions of different etiology |
| Fibroblasts from hearts with deletion of specific integrins |
| Detailed cardiac physiology in: |
| Normal situation |
| Pathological states such as *e.g.* hypertension, infarction |
| Hearts with deletion of specific integrins |

concomitant with an observation of higher heart weight to body weight ratios in these animals.

## 10. Fluid balance

Myocardial edema impairs cardiac function and will also over a few weeks change the composition of the collagen in the heart [5]. Acute myocardial edema in dogs induced by coronary sinus ligation causes decreased compliance and shifted the pressure volume curve of the heart. With chronic myocardial edema induced by pulmonary artery binding the primary type of collagen in the heart changed from collagen I to collagen III. In the chronic group collagen content increased from 3.9 to 5.8 mg/100 mg dry tissue weight. In non-edematous hearts collagen III was 11% of the total collagen and increased to about 90% in chronic edema while the percentage of collagen I decreased from 85% to about 10%.

In an earlier study, Davis et al. [2] demonstrated that chronic myocardial edema resulted in development of interstitial myocardial fibrosis. The edema was followed by a fibrosis where collagen I and III and prolyl-4-hydroxylase were elevated already after 3 days of chronic edema. Right ventricular collagenase activity was elevated after 3 days and decreased in left ventricle after 7 days. The edema and fibrosis has subsequently effects on the cardiac cycle since the stiffer myocardium operates at a higher normalized left ventricular end-diastolic pressure compared to control. The curve is also steeper than for the controls [5].

## 11. Growth factors

During inflammation and repair in the myocardium the same growth factors and cytokines that have effects in the loose connective tissues elsewhere are also active in the heart. As an example TGF-β is markedly induced and rapidly activated in the infarcted myocardium [54].

## 12. Future directions

Collectively the data presented herein point to that inflammatory cells and soluble mediators such as IL-1, TNF-α and PGE1/E2 can initiate an edema response in the connective tissues. This response most likely constitutes an intrinsic part of the innate immune system directly responsible for the tumor component of the classical signs of an inflammatory response as described in classical antique sources to be dolor, rubor, calor and tumor. This edema response would be functionally relevant in that it would enable an increased diffusion of defence plasma proteins and migration of egressed inflammatory cells into the affected tissue and thereby help the clearing of invading microbes. At the same time a process is initiated that will balance the edema response. Our data suggest that these processes involve the PDGF system that act upstream of the $\alpha_v\beta_3$ integrin. In addition, the edema response should be followed by a wound healing response that, if the inflammation is persistent, will lead to fibrosis. Since it has been shown in the heart that edema formation eventually leads to fibrosis and impairment of function it is of importance to study the edema responses in heart in detail, the importance of causative factors as well as the molecular mechanisms involved. Also, knowledge of molecular mechanisms involved in the transition from an initial edema response to fibrosis are important to elucidate in order to be able to intervene in the processes and reduce the risk for sustained damage to the heart muscle with subsequent functional derangement. In order to define these mechanisms a combination of *in vitro* systems such as collagen-gel contraction and studies in heart tissue has to be made much in a similar way the processes are being characterized in dermal loose connective tissue and in carcinoma (Table 1).

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