Transcapillary exchange: role and importance of the interstitial fluid pressure and the extracellular matrix

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This review will summarize current knowledge on the role of the extracellular matrix (ECM) in general and on the interstitial fluid pressure (Pif) in particular with regard to their importance in transcapillary exchange. The fluid volume in the interstitial space is normally regulated within narrow limits by automatic re-adjustment of the interstitial hydrostatic and colloid osmotic pressures in response to perturbations in capillary filtration and by the lymphatics. Contrary to this commonly accepted view, Pif can become an active force and create a fluid flux across the capillaries in several inflammatory reactions and trauma situations rather than limit the changes occurring. The molecular mechanisms involved in the lowering of Pif include the release of cellular tension exerted on the collagen and microfibril networks in the connective tissue via the collagen-binding β1-integrins, thereby allowing the glycosaminoglycan ground substance, which is normally underhydrated, to expand and take up fluid. Several growth factors and cytokines, including the platelet-derived growth factor BB, are able to reverse a lowering of Pif and restore the normal compaction of the ECM. The magnitude of the lowering of Pif varies with the inflammatory response. In several inflammatory reactions, a lowering of Pif to −5 to −10 mmHg is seen, which will increase capillary filtration by 10–20 times since the normal capillary filtration pressure is usually 0.5–1 mmHg (skin and skeletal muscle). Unless this lowering of Pif is taken into account, the enhanced solute flux resulting from an inflammatory response will be ascribed to an increased capillary permeability.

Keywords Connective tissue • β-Integrin receptors • Glycosaminoglycans • Inflammation

1. Introduction

Loose connective tissues are an integral part of all organs. They harbour the vast majority of the interstitial fluid volume and surround peripheral blood vessels. The traditional view on the control of the interstitial fluid volume is that it is regulated within narrow limits, and acts as a sink or storage space for the fluid filtered across the capillary wall.1 We will summarize the current knowledge that (in part) contradicts the traditional view on the role of the loose connective tissues in transcapillary fluid exchange. We will focus on conditions where these tissues can take on an ‘active’ role in filtering fluid across the microcirculation via modulations of the interstitial fluid pressure (Pif). Pif is the pressure that can be measured in a fluid-filled catheter inserted into the tissue and connected to a pressure transducer and acts as filling pressure for the initial lymphatics1 in the absence of other external forces such as muscle contractions. Pif is one of the four pressures determining transcapillary fluid flux and as such, it also contributes to determine transcapillary solute flux via its influence on this fluid flux.

Our concept for the control of Pif places a dynamic and active pressure on the outside of the capillary and includes dynamic cellular control of the tension exerted on the extracellular collagen and microfibrillar networks2,3 via collagen-binding β1-integrins.1 This is in addition to Pif being a function of the interstitial volume as described by interstitial compliance.1 In this review, we discuss situations where increased transcapillary fluid flux originates from lowered Pif, and how the lowering of Pif may contribute to other factors, in particular an apparent altered capillary permeability, since the increased fluid flux will increase convective solute transport across the capillary wall. Therefore, this review should in particular be seen in the context of the review of Levick and Michel5 on transcapillary fluid flux, that of Curry and Adamson6 on the regulation of capillary filtration coefficient and, that of Bates7 on effects of growth factors [or VEGF (vascular endothelial growth factor)] on transcapillary permeability and exchange. A recent review by us1 was one of several that had a focus on cardiac function and fibrosis.8 The present review has a general focus on the normal physiology of transcapillary exchange and how...
cellular interstitial cells and their signalling can modify transcapillary exchange of fluid and protein across the microcirculation.

2. The loose connective tissues: the composition of the extracellular matrix

Loose connective tissues are an integral part of all organs of the body. Typically, an organ consists of parenchymal cells, the circulatory system (including the lymphatic system), and the loose connective tissues that constitute the framework in which the circulatory system is embedded. Although principally similar in all organs, there are distinct differences in the composition and relative amount of the different constituents between tissues. Finally, pathological conditions such as chronic inflammation or a cancer can alter the composition and function of the circulatory system and the loose connective tissue within an organ.

Typically, the extracellular matrix (ECM) of loose connective tissues consists of four principal components. First, there is the fibrous collagen network creating the scaffold that organizes the three-dimensional structure in which the blood vessels of the organ are embedded and that forms a scaffold for the basal membranes to which parenchymal cells are attached. Second, there is the microfibril-elastin fibrous system. Third, there is the ground substance formed from proteoglycans, composed of glycosaminoglycans (GAGs) covalently linked to a core protein and the free glycosaminoglycan hyaluronan. GAGs are long unbranched amino-sugar polymers, which interact with the fibrous components of the ECM but may also occur freely and unbound in interstitial fluid. The ECM provides the route of transport for nutrients and waste products and determines the physical transport properties (e.g. hydraulic conductivity, compliance) of the tissue. Finally, the interstitial fluid is an ultrafiltrate of plasma with a total protein concentration of \( \approx 50–60\% \) of that in plasma and with an electrolyte composition similar to that of plasma. The composition of the interstitial fluid with respect to proteins in plasma is a function of the fluid flux across the capillary, the size and charge of the molecule in question, as well as the barrier properties of the capillary wall.

Several of the structural components of the ECM can be ‘washed out’ from the interstitium by raising the fluid flux. In the synovium, the concentrations of chondroitin sulfate and heparan sulfate content fell to \( \approx 70\% \) of control with increasing fluid flux, whereas that of hyaluronan remained constant. Furthermore, in the intestine, similar observations have been made for hyaluronan. In these two tissues, the data collectively suggest that hyaluronan synthesis is increased with increased removal to keep the interstitial concentration constant. Likewise, in the renal medulla, the concentration and content of hyaluronan is subject to large and rapid changes (hours) where waterloading in normal rats nearly doubled hyaluronan in medulla, but not in cortex. This increase in hyaluronan was not seen in experimental diabetes with water-loading. Thus, rather than being a structure with a constant composition that is turning over at a slow rate, at least hyaluronan turns over rapidly and synthesis rate can be changed in a relatively short time. This occurs either as a function of the enhanced fluid flux and by ‘washing out’ the individual matrix components, or by rapidly occurring de novo synthesis as a response to altered interstitial fluid flux or concentration. As is evident in the renal medulla, which is likely studied in the most detail from an integrated perspective, the enhanced fluid flux sets off enhanced synthesis, most likely via hyaluronan synthase 2.

3. Biophysical properties of the ECM

A comprehensive review on the physiological properties of ECM was written by Levick more than 20 years ago. This review provides an extensive comparison of available data towards modelling predictions, to a large extent based on Darcy’s law. The field has not gained much with regard to new data on interstitial transport properties since then, and it seems that much benefit could have been made from the progress made in molecular biology and the large number of transgenic animal models with altered ECM. One example on how such an approach may give new insight is the recent study of Schwartz and co-workers in which the authors measured hydraulic conductivity based on infusion of saline into the tissue in two mouse models of primary lymphoedema, the Chy mouse, and the K14-VEGF-3-Ig mouse. The K14-VEGF-3-Ig mouse had greatly increased dermal conductivity, but neither their controls nor the Chy mice displayed such an increase.

With regard to mathematical modelling for understanding interstitial transport, several detailed models of transport properties have been developed. As one example, Yao and Gu have modelled complex transport phenomena in the ECM of cartilage as a charged porous fibrous material including diffusive and convective solute fluxes in porous fibrous media and a continuum mixture theory. They included the effect of mechanical loading on solute diffusion under unconfined compression with a frictionless boundary condition using numeric analysis. As another example, Olsen et al. developed modelling frameworks for studying dynamic anisotropy in connective tissue based on partial differential equations operating on a macroscopic scale and including cells. The model has been expanded to two matrix types and the results are robust and consistent with experiments representing cells as discrete objects in a continuum of ECM. It is the latter type of modelling that would seem to be required to account for the stresses that, in our mechanistic model, contribute to the ‘active’ control of \( P_e \).

4. Normal exchange across the capillary wall

The normal exchange across the capillaries, which is determined by the transcapillary hydrostatic and colloid osmotic pressures, is the focus of the review by Levick and Michel in this issue. The skin and skeletal muscle jointly contain around two-thirds of the extracellular fluid. In a human with an extracellular fluid volume of 15 L, the plasma volume is 3 L and the total lymphatic return is \( \approx 3–5 \text{ L per day} \). It is generally accepted that a volume nearly double that enters the initial lymphatics, but about half the amount entering the initial lymphatics is absorbed in the lymph nodes. Furthermore, the contribution to the overall lymphatic return is larger from the intestinal organs than from skin and skeletal muscle. Taken together, the available evidence suggests that the interstitial fluid in skin and skeletal muscle is turned over in \( \approx 24–48 \text{ h} \).
The fluid flux across the microcirculation \((J_v)\) is described according to Starling’s hypothesis as:

\[
J_v = CFC(P_c - P_t - \sigma(COP_p - COP_if)) = CFC \Delta P,
\]

where \(CFC\) is the capillary filtration coefficient, \(\Delta P\) is the product of the net filtration pressure, and \(P_c\) and \(P_t\) are hydrostatic and colloid osmotic pressures, respectively. Furthermore, the subscripts \(c\), \(p\), and \(if\) denote capillary, plasma, and interstitial fluid, respectively, and \(\sigma\) is the reflection coefficient for plasma proteins across the capillary. \(P_t\) in skin and skeletal muscle is around ambient pressure or slightly sub-atmospheric, i.e. around \(-1\) mmHg.1

In man, plasma colloid osmotic pressure is 25–28 mmHg and \(~60%\) of this value in the interstitial fluid of skin and skeletal muscle. In experimental animals, the \(COP_p\) is \(~20\) mmHg and again the \(COP_if\) is \(~60%\) of that in plasma in skin and skeletal muscle in rats and mice.20–21 Levick and Michel5 show that colloid osmotic pressure immediately distal to the primary endothelial barrier (e.g., the filtrate beneath the glycocalyx) may not equal interstitial osmotic pressure. In the steady state, this difference may be small but it may be large during rapid filtration.

The review by Levick and Michel5 argues that, in steady state, there is only filtration in the microcirculation, as opposed to filtration at the arterial end and not re-absorption at the venous end. The important extension of this discussion for the focus of this review is not whether re-absorption actually takes place normally, but rather that the trans-capillary net filtration gradient required to generate the normal net capillary filtration is \(~0.5–1\) mmHg. This pressure is usually calculated as pre-nodal lymph flow divided by \(CFC\) since in a steady state, the net capillary filtration \((J_c)\) will equal the pre-nodal lymph flow \((J_l)\) and then Eq. (1) can be rewritten as:

\[
\Delta P = \frac{J_v}{CFC} = \frac{J_l}{CFC} \quad \text{since} \ J_v = J_l
\]

or

\[
CFC = \frac{J_v}{\Delta P} = \frac{J_l}{\Delta P}.
\]

From this equation, it becomes clear that if \(CFC\) is estimated from lymph flow or capillary filtration, most commonly measured as increased weight (using isogravimetric methods) and estimating the alterations in \(\Delta P\) from the events in the circulation alone, an overestimation of the change in \(CFC\) will result since \(\Delta P\) will be underestimated unless also accounting for a lowering of \(P_t\). The clearest example of how complex this situation may be is that a net capillary pressure of \(~250–300\) mmHg was calculated by Artursson and Mellander22 to be required in addition to an independent measured rise in \(CFC\) of about two to three times above control to account for the fluid accumulation in burn injury.22–24 If both factors are not considered simultaneously to account for the oedema formation, one may well be misled to ascribe events in the loose connective tissues to changes taking place at the capillary wall. The large increase in net filtration pressure in burn injury was subsequently explained from a lowering of \(P_t\).25,26

\section*{5. Normal control of interstitial fluid volume vs. oedema}

The interstitial fluid volume is normally kept within tight boundaries by automatic re-adjustments of the transcapillary fluid flux when


There are four factors and issues that are important in the long-term control of the interstitial volume, i.e. hours and beyond. First, there is the interstitial compliance as defined in Eq. (4) linking interstitial volume (IFV) and pressure:

\[
\text{Compliance} = \frac{\Delta P}{\Delta \text{IFV}}.
\]

Compliance around control is \(~14%\) in skin and skeletal muscle that are not enclosed in fascias, i.e. an increase in the interstitial volume of \(14%\) will raise \(P_t\) by 1 mmHg. When interstitial volume increases above \(50%\) of control, compliance increases dramatically so that even with a large increase (>2–300% of control) and long-standing oedema, \(P_t\) is only a few mmHg. However, interstitial compliance may not be constant. In acute oedema formation following freeze injury, there can be substantially higher \(P_t\) as seen from an increase in \(P_t\) from \(-1\) to 25 mmHg in 60 min as oedema developed to 2–300% above control.27 After placing a tourniquet and limiting further fluid accumulation, \(P_t\) fell in the subsequent hour to below 10 mmHg even when maintaining interstitial volume, implying that the compliance must increase simply due to overstretching by the increased interstitial volume.27

Second, at a compliance of \(14%\), an increased capillary filtration will cause an increase in \(P_t\) and a fall in \(COP_if\), which are of similar magnitudes and in turn act across the capillary wall to limit further filtration. Similar but opposite changes are seen upon re-absorption. These automatic re-adjustments of \(P_t\) and \(COP_if\) have been called ‘auto-regulation’ of capillary filtration by the interstitial pressures.1

Third, \(P_t\) and/or interstitial volume act as the filing pressure for the initial lymphatics to increase lymph flow and limit oedema formation. Fourth, interstitial exclusion, i.e. the property that the plasma proteins do not have access to the full interstitial volume due to steric exclusion by the structural macromolecules,28 will influence the time to
reach a new steady state, not the steady state itself. Thus, a higher exclusion results in reduced time to reach a new steady state since there are less plasma proteins in the loose connective tissue.1

On the road to a new steady state resulting from increased filtration, lymph flow will increase until a steady state is achieved where lymph flow and protein flux again equal the corresponding net capillary fluxes and there will be a relatively larger flux of protein out of the tissue compared with the influx across the capillary. The reason being that the interstitial fluid protein concentration in the new steady state is lower and the enhanced removal of protein with lymph ‘washdown’ reduces the protein content, not merely dilutes it. In turn this allows the new steady state to be achieved by less expansion of the interstitial volume than would otherwise be the case.

7. Control of total extracellular fluid volume

The control of the interstitial volume is normally linked to the renal control of the total extracellular volume. Control via the kidney and the central nervous system seems sufficient to control the extracellular volume since the transcapillary control of microvascular exchange exhibits such strong ability to ‘auto-regulate’ the interstitial volume.

However, a novel concept of connective tissue control of the interstitial fluid volume and sodium uncoupled from total extracellular volume has been suggested for tissue macrophages in skin through accumulating sodium in salt-sensitive hypertension, and without being accompanied by the accumulation of water.29 A high-salt diet (HSD) in rats has been demonstrated to cause interstitial hypertonic sodium accumulation in skin with increased density and hyperplasia of the initial lymphatic capillary network, with sodium likely binding to GAGs.29

The mechanisms involved include the activation of tonicity-responsive enhancer-binding protein (TonEBP) in the mononuclear phagocyte system (MPS) cells in the loose connective tissues. The TonEBP binds the promoter of the gene encoding VEGF-C to induce VEGF-C secretion by macrophages. A depletion of the MPS or trapping VEGF-C is suggested to increase interstitial hypertonic volume retention and furthermore decrease endothelial nitric oxide synthase expression resulting in increased blood pressure in response to the HSD. It is suggested that VEGF-C acts as control for osmosensitive and hypertonicity control in the regulation of blood pressure.29

An increased amount of sodium would have to be bound to the negatively charged GAGs or to be localized intracellularly. However, the former would require either an increased amount of negative charge on the GAGs or altered electrolyte composition in interstitial fluid, and thereby also plasma. A more likely explanation would then seem to be increased intracellular content of sodium in the connective tissue cells. The principal importance of this study is nevertheless to point towards a novel role for the connective tissue and its cells in the handling of sodium. This mechanism points towards the control of sodium balance as being, at least in part and under certain circumstances, uncoupled from renal control of extracellular fluid volume.

8. Fluid exchange in acute inflammation

Acute inflammatory reactions are frequently associated with rapid and large changes in the transcapillary exchange of fluid and protein fluxes. When oedema in skin associated with acute inflammation occurs in <10 min, there has been at least a doubling of the interstitial volume during the same time period. Since it normally takes 1–2 days to filter this amount of fluid across the capillary, the fluid flux in the most extreme cases as seen in a burn injury must have increased by ~200–300 times above the normal control. CFC is measured to increase by two to three times above normal, leaving the net filtration pressure to explain the largest part of the increase in capillary filtration. The increase in net filtration pressure is calculated at 250–300 mmHg and is higher than what can be generated from the circulatory system. Surprisingly, a lowering of $P_f$ was seen concomitant with oedema formation rather than an increase as commonly assumed when oedema forms. Lowering of $P_f$ to as much as ~150 mmHg was observed in these initial studies and was associated with physical denaturation of the collagen.24–26,30

Several inflammatory agents, cytokines, and chemokines have been studied for the same effect on $P_f$ and a lowering, rather than an increase in $P_f$, has been seen for a large number of substances as oedema developed. Although the most pronounced lowering was seen after freezing injury27 and burn injury,26 both inflicting a physical injury to the tissue, several inflammatory mediators and pathological conditions associated with oedema formation have been demonstrated to have a lowering of $P_f$ as part of the pathophysiology. These conditions included, among others, anaphylaxis induced by degranulating mast cells with compound 48/80 (C48/80),31–33 platelet-activating factor,34 acute asthma induced by antigen exposure to rats sensitized to egg albumin,35 neurogenic inflammation following the stimulation of the vagal nerve36 and also by LPS77 and tumour necrosis factor-$\alpha$ (TNF-$\alpha$).38 Taken together, the range of conditions where a lowering of $P_f$ occurred as part of the pathological processes suggested that several mediators that induced a local inflammation could also elicit a lowering of $P_f$. Some of the mediators tested include prostaglandins $E_1$, $E_2$, and $I_2$,39 IL-1β and IL-6,38 and the phosphatidylointhol 3-kinase inhibitors wortmannin and LY294002.39

Each case demonstrates that the lowering of $P_f$ is important as a phenomenon by itself as well as demonstrating that the loose connective tissue can ‘actively’ enhance the capillary filtration.

It should be emphasized that $P_f$ will contribute to the transcapillary fluid flux in the acute part of an inflammation. As soon as a new steady state is achieved between the tension in the fibre networks, the swelling ground substance and $P_f$, the oedema, and increased capillary filtration will have to be maintained by increased capillary hydrostatic pressure and increased capillary permeability (increased CFC and lowered capillary reflection coefficient).

9. Maintenance of an inflammatory oedema. The capillary reflection coefficient

The lowering of $P_f$ will contribute in the initial phase of the inflammatory reaction to the rapid and initial flux across the microcirculation to establish the inflammatory oedema. It should be noted [cf. Eq. (1)] that a lowering of $\sigma$ contributes to the increased capillary filtration by lowering the total colloid osmotic pressure gradient acting across the capillary not only during the acute phase but also at later stages of the inflammation. As the oedema develops, $P_f$ will attain positive values and the maintenance of the oedema depends
upon an increased capillary pressure (from the vasodilation) and a lowered α.

10. Integrins and $P_{if}$: antibodies to $\beta_1$- and $\alpha_2\beta_1$-integrins

Insight into the molecular mechanisms involved in the control of $P_{if}$ was obtained by parallel studies using the collagen gel contraction assay and in vivo experiments. In this assay, typically 100,000 fibroblasts are mixed with soluble native collagen at neutral pH, and gels are formed at 37°C. The rate at which the cells compact the collagen gel is a function of the force by which the cells act on the collagen through integrins that anchor cells to the collagen fibres.51 The integrins are transmembrane heterodimeric adhesion receptors mediating cell–cell and cell–matrix adhesion and are built from an $\alpha$- and a $\beta$-subunit and capable of inside-out an outside-in signalling.42 There are four collagen-binding integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$, and $\alpha_{11}\beta_1$ with $\alpha_2\beta_1$ and $\alpha_1\beta_1$ that bind native fibrillar collagen.43,42 A role for the $\alpha_2\beta_1$-integrin in rats in the lowering of $P_{if}$ was established in early studies since subdermal injection of a monoclonal antibody to this integrin will lower $P_{if}$ and cause oedema formation while a control IgG was without effect.52 There have been in vivo studies in parallel with in vitro experiments using the collagen gel contraction assay. Thus, once an agent has been shown to lower the rate of collagen gel contraction in vitro, it has also been shown to cause lowering of $P_{if}$ and oedema in vivo. Recently, it has also been established that the $\alpha_1\beta_1$-integrin also has a role similar to that previously described for the $\alpha_2\beta_1$-integrin.33

Having established that the $\alpha_2\beta_1$-integrin was involved in lowering $P_{if}$ Lidén et al.32 demonstrated that the reversal of a lowered $P_{if}$ by platelet-derived growth factor (PDGF)-BB,46 (see what follows) exerted its effect via the $\alpha_2\beta_1$-integrin. This conclusion and the novel function of the $\alpha_2\beta_1$-integrin were based on two lines of evidence. First, specific anti-$\beta_2$-integrin IgG blocked PDGF-BB-induced collagen gel contraction in cells lacking functional collagen-binding $\beta_1$-integrins or when the latter were perturbed. Second, this observation was confirmed in vivo since PDGF-BB had no effect in $\beta_1$-integrin null mice, i.e. the growth factor was not able to normalize $P_{if}$ in the dermis of such animals. Thus, we therefore suggest that under normal conditions, tension from the cells to the dermal fibres is maintained by $\beta_1$-integrin-mediated contraction. Pro-inflammatory mediators will release the $\beta_1$-integrins to result in lowering of $P_{if}$ and oedema formation, whereas PDGF-BB will counteract the tendencies to oedema through stimulating the activity of the $\alpha_2\beta_1$-integrin.

11. Reversal of an already lowered $P_{if}$

In the collagen gel compaction assay, several agents will raise the speed of compaction. In particular, the effect of PDGF has been studied and was typically administered subcutaneously after an anaphylactic reaction or other inflammatory reaction had been induced by i.v. dextran. PDGF-BB raised $P_{if}$ in a dose-dependent manner, whereas both PDGF-AA, which specifically binds and stimulates the $\alpha$-receptor, and fibroblast growth factor were without effect, clearly demonstrating specificity.44 Subsequently, insulin,45,46 prostaglandin F2α,39 and $\alpha$-trinositol48 have also been demonstrated to reverse a lowering of $P_{if}$. In addition, large doses of vitamin C47 and $\alpha$-trinositol48 attenuated the lowering of $P_{if}$ in burn. Furthermore, corticotropin-releasing factor and mystixins have well-documented effects in limiting the oedema associated with the inflammatory response towards several inflammatory agents and do this at least in part via attenuating the lowering of $P_{if}$ occurring as part of the inflammatory response.49

12. Involvement of the cytoskeleton

In relation to microvascular exchange, the cytoskeleton will influence both the function of the endothelial cells and $P_{if}$. Cytochalasin D disrupts actin; it lowered $P_{if}$ and caused oedema,50 whereas both nocardazole (depolymerizes microtubules) and colchicine (inhibits tubulin polymerization) were without effect on $P_{if}$ and oedema formation.50 Phalloidin caused fixation of the actin and had no effect on $P_{if}$ and oedema formation but attenuated the effects of taxanes paclitaxel and docetaxel, which both caused fixation of microtubule, and when given alone lowered $P_{if}$ and caused oedema.51 These observations are in agreement with the model outlined in what follows where the expanding glycosaminoglycan gel is restrained by cellular tension and the release of this tension allows the tissue to expand, in turn lowering $P_{if}$. Furthermore, the lowering of $P_{if}$ in dextran-induced anaphylaxis was abolished by phalloidin.52 Altogether, the cytoskeleton can modify $P_{if}$, and thereby attenuate or enhance an inflammatory oedema.

Phalloidin will also attenuate oedema formation in other models of inflammation. As two examples, phalloidin prevented bradykinin-induced leucocyte/endothelial cell-adhesive interactions and leucocyte migration,53 and oedema induced by ischemia-reperfusion is prevented by treatment with phalloidin.54 The referenced studies point to complex effects on several cell types after pharmacological interference with the cytoskeleton. This, in turn, requires that both interstitial changes in pressures and endothelial alterations are analysed separately when the overall effects is to be outlined.

13. Mechanistic model for dynamic control of $P_{if}$

We have suggested the following mechanistic model for the cellular control of $P_{if}$ based on experiments with agents that induce a lowering of $P_{if}$ or attenuation of the lowering of $P_{if}$ as well as the studies on integrins, growth factors, cytokines, and chemokines. First, when loose connective tissues are excised and immersed in isotonic saline, they will swell due to the properties of the ground substance. Interstitial pressure and extracellular matrix changes in pressures and endothelial alterations are analysed separately when the overall effects is to be outlined.
capillary filtration until a steady state is reached between the forces in the fibre networks, the ground substance, and \( P_s \) where the interstitial volume again is constant and capillary filtration again equals lymph flow.

### 14. Intracellular signalling pathways

An important issue for the studies of intracellular signalling has been to compare the in vivo studies in mice and rats and the in vitro studies using fibroblasts in the collagen gel compaction assay. The studies underlining the mechanistic model for cellular control of \( P_s \) clearly demonstrate that the cytoskeleton can influence \( P_s \) and have been based on a combination of the in vitro collagen gel contraction assay and in vivo animal studies. This same approach was used in studies where we demonstrated that under normal homeostasis the collagen-binding integrin \( \alpha_\text{IIb}\beta_3 \) is of particular importance for the control of \( P_s \) in rat dermis as well as to mediate contraction of collagen gels by cultured cells.\(^{4,5,5,5}\)

Principally, two different classes of substances have been tested. First, those that like PDGF induced contraction of fibroblasts in the collagen gel compaction assay. The signalling by PDGF-BB involves signalling via phosphatidylinositol 3'-kinase activity. Furthermore, the migratory response induced by PDGF-BB induces reorganization of the actin cytoskeleton and has been abolished or attenuated by inhibitors of phosphoinositide-3-kinase and protein kinase C.\(^6\) The involvement of PDGF-BB has been demonstrated in several in vivo studies, including the reversal of an already lowered \( P_s \) in anaphylaxis.\(^{4,6}\) Based on in vivo experiments in transgenic mice and in vitro experiments, we have demonstrated that the reversal of a lowering of \( P_s \) by PDGF-BB\(^8\) is dependent on phosphatidylinositol 3-kinase (PI3K) since transgenic mice with mutated PDGF \( \beta \)-receptors that cannot activate PI3K after ligand stimulation with PDGF-BB were unable to restore \( P_s \) in response to PDGF-BB.\(^9\)

The second group is pro-inflammatory substances that attenuate the contraction rate in vitro and also lower \( P_s \) in vivo. The involvement and also the specificity of the prostaglandins have been demonstrated where prostaglandins \( E_1 \), \( E_2 \), and \( I_2 \) will attenuate the collagen gel compaction, whereas prostaglandin \( F_2 \alpha \) will enhance it.\(^{10}\) The inhibition of collagen gel compaction seems to result from signalling pathway(s), resulting in intracellular concentrations of cAMP.\(^{11,12}\) This is in line with the observation that intradermal injection of cAMP alone will attenuate a lowering of \( P_s \).\(^{5,5}\)

Also, pro-inflammatory cytokines, e.g., IL-1 and TNF-\( \alpha \) and IL-1, will inhibit fibroblast-mediated collagen gel contraction.\(^{13}\) Prostaglandins have been demonstrated to act as second messengers for the inhibitory effects of IL-1 and TNF-\( \alpha \) on contraction,\(^{14}\) and prostaglandins \( E_1 \), \( E_2 \), and \( I_2 \) inhibit fibroblast-mediated collagen gel contraction.\(^{15}\) cAMP has been suggested to interfere with collagen gel compaction via phosphorylation of myosin light chain kinase.\(^{16,17}\)

The intracellular signalling pathways involved in the control of \( P_s \) are complex as outlined earlier, with the signalling via phosphatidylinositol 3'-kinase activity and PDGF-BB as central and important focal points.\(^{18}\)

### 15. Future directions

The loose connective tissues can ‘actively’ alter transcapillary fluid flux via active cellular tension on the collagen and microfibril networks. Inflammatory cells and soluble mediators such as IL-1, TNF-\( \alpha \), and prostaglandins \( E_1 \), \( E_2 \), and \( I_2 \) can initiate an oedema response which likely is an intrinsic part of the innate immune system directly responsible for the tumour component of the classical signs of an inflammation. The oedema response in inflammation would be functionally relevant in that it would enable an increased diffusion and convection of defence plasma proteins and migration of egressed inflammatory cells into the inflamed tissue. At the same time, a process is initiated that will balance the oedema response. Our data suggest that these processes involve the PDGF system that acts upstream of the \( \alpha_\text{IIb}\beta_3 \)-integrin. In addition, a wound-healing response that, if the inflammation is persistent, will lead to fibrosis should follow the oedema response. Knowledge of molecular mechanisms involved in the transition from an initial oedema response to fibrosis is important to elucidate in order to be able to intervene in the processes and reduce the risk for sustained damage and functional derangement. In order to define these mechanisms, a combination of in vitro systems such as collagen gel contraction and tissue has to be made.

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