Fluid pressure in human dermal fibroblast aggregates measured with micropipettes

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Stuhr, L. E. B., A. Reith, S. Lepsøe, R. Myklebust, H. Wiig, and R. K. Reed. Fluid pressure in human dermal fibroblast aggregates measured with micropipettes. Am J Physiol Cell Physiol 285: C1101–C1108, 2003.—Previous studies indicated that connective tissue cells in dermis are involved in control of interstitial fluid pressure (Pif). We wanted to develop and characterize an in vitro model representative of loose connective tissue to study dynamic changes in fluid pressure (Pf) over a time course of a few minutes. Pf was measured with micropipettes in human dermal fibroblast cell aggregates of varying size (<100- and >100-μm diameter) and age (days 1–4) kept at different temperatures (–15, 25, and 35°C). Pressures were measured at different depths of micropipette penetration and after treatment with prostaglandin E1 (PGE1), latanoprost (PGF2α), and ouabain. Pf was positive (more than +2 mmHg) during control conditions and increased with increasing aggregate size (day 2), age (day 4 vs. day 1), temperature, and depth of micropipette penetration. Pf decreased from 2.9 to 2.0 mmHg during the first 10 min after application of 10 μl of 1 mM PGE1 (P < 0.001). Pf increased from 3.0 to 4.8 mmHg (P < 0.01) after administration of 10 μl of 1.4 μM ouabain and from 3.1 to 4.4 mmHg after addition of 5 mM PGF2α (P > 0.05). In conclusion, we have developed and validated a new in vitro method for studying fluid pressure in loose connective tissue elements with the advantage of allowing reliable and rapid screening of substances that have a potential to modify Pf and studying in more detail specific cell types involved in control of Pf. This study also provides evidence that fibroblasts in the connective tissue can actively modulate Pf.

INTERSTITIAL FLUID PRESSURE (Pif) is one of the determinants of transcapillary fluid exchange and plays an important role in controlling interstitial fluid volume (24). In previous studies Pf was measured in different animal tissues with micropuncture after the administration of test substances either locally or systemically (24). Pf in skin is normally approximately –1 mmHg (36), whereas during burn injury and inflammation Pf is lowered substantially and hence becomes an important contributor to increasing capillary filtration and edema formation (17, 18, 25). The mechanisms that generate such changes in Pif are presently not fully understood.

During recent years our group has demonstrated that the cells of the connective tissue can influence the physical properties of the extracellular matrix. They actively participate in transcapillary fluid exchange by altering Pf and hence play an important role in controlling interstitial fluid volume (24). Meyer (19) showed that connective tissue, when allowed free access to fluid, swelled because of its content of glycosaminoglycans and hyaluronan. This tissue swelling was counteracted by the collagen and microfibrilll networks. Dermal fibroblasts cultured in three-dimensional collagen gels contract the gel (1, 2, 20), indicating that the connective tissue cells exert tensile forces. The contraction has been shown to be dependent on the collagen-binding β1-integrin, one of eight subfamilies of β-integrins (10, 11, 15, 26, 29).

Fibroblasts are the predominant cell type in the connective tissue. They are responsible for synthesis of collagen, elastic fibers, and the complex carbohydrates of the connective tissue (12). The fibroblast cells within an aggregate are held together by cell processes, cell adhesion molecules, extracellular matrix proteins, and a variety of cell-cell junctions (32).

The most commonly used in vitro system used to study cell-matrix interaction is the collagen gel contraction assay, in which ~100,000 fibroblasts are mixed with α-helical collagen. Within 24 h the fibroblast compacts the gel to ~10% of its original volume. Agents that act on the cell-matrix contact or the rate of compaction raise or slow the rate of compaction with an effect that is detectable in the course of hours. The aim of this study was therefore to develop, and thereafter characterize, an in vitro model of loose connective tissue using human dermal fibroblast cell aggregates to study the dynamic changes in pressure in the aggregates over a time course of a few minutes.

METHODS

Preparation of Human Dermal Fibroblasts

Human skin (5 × 5 cm) was obtained from patients undergoing plastic surgery for stomach reduction. The patients

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gave their informed consent, and approval of the study was given by the Regional Ethical Committee. The skin was washed in sterile phosphate-buffered saline (PBS) and transferred to a sterile petri dish, where the subcutaneous fat was removed by dissection. Thereafter, the skin was cut into very fine pieces (<1 mm²) and suspended in a minimal amount of Dulbecco’s modified Eagle’s medium (DMEM; PAA Laboratories, Linz, Austria). This medium was supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 1% l-glutamine (Life Technologies, Paisley, UK) and seeded in a 75-cm² tissue culture flask (Nunc, Nalga Nunc International). The tissue explants were cultured without disturbance until fibroblast cell outgrowth was detected. The medium was replenished at this stage, and the cells were subcultured when significant cell growth was observed and grown to confluence. Primary cultures of fibroblasts between passages 1 and 8 were used. All cells were cultured under standard conditions at 37°C in a humidified atmosphere of 5% CO₂-95% air.

Preparation of Cell Aggregates

Cell aggregates were prepared with human dermal fibroblasts at passages 2–8. Three-dimensional aggregates were prepared according to the method of Yuhas et al. (34). Cell culture flasks (25 cm²) were base coated with 0.75% agar in 5 ml of DMEM. Fibroblasts were seeded at a density of 3 × 10⁶ cells per 25-cm² flask. The cells were cultured without disturbance until the cell aggregates formed.

Transmission Electron Microscopy

Human dermal fibroblast cell aggregates were cultured, carefully placed in a test tube, and fixed in 2% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2). They were rinsed in 0.1 M cacodylate buffer (2 × 15 min), postfixed in 1% osmium tetroxide for 35 min, and dehydrated in a series of ascending concentration gradients of ethanol. Specimens were then embedded in araldite plastic. The semithin sections were stained with 1% toluidine blue. The ultrathin sections were double stained with 1% uranyl acetate (20 min) and lead citrate (10 min) before being viewed in a JEOL 100CX transmission electron microscope.

Measurement of Fluid Pressure

Fluid pressure (Pₖ) in the three-dimensional cell aggregates was measured with a micropuncture system. A borosilicate glass pipette (GC 100–15, 1-mm OD × 0.58-mm ID) was pulled on a micropipette puller (P-87; Sutter Instruments). The tip was sharpened to a diameter of 3–9 μm. The pipette was filled with 0.5 M NaCl solution colored with Evans blue dye. The pipette was then connected to a micromanipulator (Leitz) and a servo-controlled counterpressure system (36). Calibration was performed before each experiment. Zero control pressure was measured in a bath of 0.9% NaCl (isotonic saline) that was set at the same height as the petri dish containing the cell aggregates to be micropunctured. Measurements of aggregate pressure (Pₖ) were performed by inserting the glass pipette directly into the central part of the fibroblast aggregate with the use of a stereomicroscope (Wild M5, Heerbrugg, Switzerland). There was no visible compression or retraction of the aggregate during the micropuncture procedure. Only aggregates that were attached to the bottom of the petri dish could be micropunctured. The following criteria had to be fulfilled for the measurements to be accepted: 1) The recorded pressure did not change when the feedback gain of the servo-controlled unit was varied. 2) After fulfillment of the first criterion, communication between the fluid in the pipette and the fibroblast cell fluid was tested by applying suction to the pipette, which should raise electrical resistance in the pipette because of the entry of fluid with a low tonicity. 3) The zero pressure measured in the bath of saline after micropuncture returned to the initial zero measurement.

Measurements

Unless otherwise stated, the measurements were performed under control conditions, defined as a medium temperature >20–24°C, cell aggregates of passages 7–8, and measurements performed in the central part of the aggregate. We have not been able to demonstrate any cell characteristics differences between passages 7 and 8 in our study. Aggregate Pₖ was also measured under the conditions described below.

Cell aggregates of different sizes. Cell aggregates <100 and >100 μm in diameter were used. The aggregate size was measured with an eyepiece graticule (Mol-93; Leica).

Cell aggregates of different ages. Aggregates were cultured and kept under standard culture conditions at 37°C in a humidified atmosphere and measured on day 1, 2, 3, or 4 to see whether age influenced Pₖ.

Different depths of penetration within the cell aggregate. To relate Pₖ to depth of penetration within the cell aggregate, long pipette advancement distances were required and pipettes were made with a long shank to minimize tissue distortion and compression artifacts at deep insertion distances. A dial indicator was mounted onto the fixed part of the micromanipulator, allowing measurement of the pipette advancement to the nearest 10 μm. With the pipette on the aggregate surface, the angle over the horizontal plane between aggregate and pipette was measured to the nearest degree and the pipette was inserted. The depth of measurement was then calculated as the distance of advancement times the sine of the angle between the horizontal plane and pipette. Three or four pressure measurements were recorded within each cell aggregate during the insertion from the surface to the center, with each recording lasting 1–3 min.

Different temperatures. The cells were taken directly from the 37°C culture incubator to the laboratory. At room temperature the bath temperature was measured at 25°C. The petri dish was placed on a bed of ice for measurements at 15°C. Measurements performed at 35°C required the petri dish to be placed on a heating plate set at 35°C. The medium temperature was monitored in the petri dish throughout the experiment with a digital thermometer. It was assumed that the temperature within the aggregate was the same as that in the surrounding medium.

Under influence of test drugs. A 2-min stable Pₖ control period was always obtained before measurements commenced. The drugs were dropped carefully onto the fibroblast cell aggregate, and the effect was studied over a period of 20–30 min. All measurements in this group were performed at room temperature, and only one aggregate was measured in each petri dish. To evaluate a possible effect of the application procedure, a control study using 10 μl of saline (0.9% NaCl; n = 3) was performed. Prostaglandin E₁ isopropyl ester (PGE₁; n = 10) was obtained from Sigma (St. Louis, MO). PGE₁ is a nonselective prostanoid EP receptor agonist and was applied at 1 μM in a volume of 10 μl. PGF₂α, (latanoprost, lithium salt; n = 10) was obtained from Sigma. Latanoprost is a selective prostanoid FP receptor agonist and was applied at 1.42 mM in a volume of 5 μl. Ouabain (n = 10)
was also obtained from Sigma. Ouabain is a \( \text{Na}^+\text{-K}^+\)-ATPase inhibitor and was applied at 1.4 \( \mu \text{M} \) in a volume of 10 \( \mu \text{l} \).

**Statistical Analysis**

One-way analysis of variance (ANOVA) with repeated measurements followed by Bonferroni’s test was used for statistical analysis. \( P < 0.05 \) was considered statistically significant. Data are given as means ± SD.

**RESULTS**

**Transmission Electron Microscopy**

The cell aggregate ultrastructure is shown in Figs. 1 and 2. Figure 1 (semithin section) shows that the cell aggregate is cohesive and almost spherical in form. The cell aggregates ranged from \(~40 \text{ to } 350 \mu \text{m}\) in diameter. Figure 2 (ultrathin section) shows that the cell aggregate was comprised of numerous fibroblast cells with many vacuoles and nuclei and endoplasmic reticulum. The intercellular spaces were frequently filled with collagenous, fibrillar, and amorphous material. The fibroblasts were connected by scattered junctions as seen in Fig. 2. A distinct extracellular basal membrane was not observed.

**Control \( P_f (n = 33) \)**

The fibroblast cell aggregate \( P_f \) was always positive and relatively high (more than \(+2 \text{ mmHg}\)) during control conditions, with an average of \(3.1 \pm 0.8 \text{ mmHg}\).

**Effect of Size on \( P_f (n = 47) \)**

There was a tendency for larger aggregates (\(>100 \mu \text{m}\)) to have a higher \( P_f \) than small aggregates (\(<100 \mu \text{m}\)). The difference in \( P_f \) related to size was statistically significant only at day 2 vs. day 1 (\( P < 0.01 \)) (Fig. 3). The average \( P_f \) was 4.6 and 2.5 mmHg in large and small aggregates, respectively, at day 2.

**Effect of Age on \( P_f (n = 47) \)**

Cell aggregates were studied over a 4-day period. There was a statistically significant effect of age on \( P_f \)
(P < 0.01), i.e., passage number, at day 4 vs. day 1 in small aggregates (Fig. 3). The average Pf was 2.5 mmHg at day 1 compared with 3.8 mmHg at day 4 in the small aggregates.

Effect of Depth on Pf (n = 36)

A significant increase in Pf was found toward the center of the fibroblast cell aggregate (Fig. 4). The average Pf was 2.4 mmHg just under the surface and 4.6 mmHg in the center of the aggregate.

Effect of Temperature on Pf (n = 46)

Increasing temperature significantly raised Pf (Table 1). The difference between the three temperature groups was statistically significant (P < 0.01). To eliminate external factors, all the aggregate groups had a similar average size and were taken from the same passages (7 and 8) and measurements were made in the center of the aggregate.

Effects of Saline, PGE1, Ouabain, and Latanoprost

The effects of saline, PGE1, ouabain, and latanoprost on aggregate Pf over a period of 20–30 min are shown in Fig. 5. There was no effect on Pf when 10 µl of saline (n = 3) was applied in an identical way as the test substances to the aggregates. Before the administration of PGE1 (n = 10), control Pf averaged 2.9 mmHg. Pf started to decrease immediately after 10 µl of PGE1 was applied. After 10 min, the Pf was −2.0 mmHg and it then started to increase again (Fig. 5A). Before treatment with PGF2α (n = 10), control Pf was 3.1 mmHg and increased rapidly after 5 µl of latanoprost

Table 1. Effect of temperature on fluid pressure in dermal fibroblasts

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Pf, mmHg</th>
<th>n</th>
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<tbody>
<tr>
<td>14.3 ± 1.9</td>
<td>1.6 ± 0.9*†</td>
<td>14</td>
</tr>
<tr>
<td>25.5 ± 0.4</td>
<td>2.9 ± 1.0†</td>
<td>19</td>
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<tr>
<td>34.7 ± 1.3</td>
<td>3.9 ± 1.0</td>
<td>14</td>
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Data are means ± SD for n fibroblast cell aggregates. *P < 0.01 vs. 25°C group; †P < 0.001 vs. 35°C group.

Fig. 5. Effects of prostaglandin (PG)E1 (1 mM, 10 µl; A), PGF2α (latanoprost; 1.42 mM, 5 µl; B), and ouabain (1.4 µM, 10 µl; C) on aggregate Pf over a period of 20–30 min. The drug was applied immediately after control measurements (0 min). Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.
were applied until it stabilized at 4–5 mmHg. It maintained this value for the following 6–20 min (Fig. 5B). Before treatment with ouabain (n = 10), control Pf averaged 3.0 mmHg. As for PGE2, pressure started to increase after the application of 10 μl of ouabain (Fig. 5C). Ten minutes after application Pf averaged 4.8 mmHg, whereas Pf returned to control values after 20 min.

DISCUSSION

In the present study we have demonstrated that human dermal fibroblast aggregates can be used as an effective in vitro model for loose connective tissue. Anchorage-dependent cells, having cell adhesion molecules on the surface, have the ability to form cell clusters or aggregates if the seeding density is sufficiently high. Fibroblast cells formed spherical aggregates (Fig. 1). In monolayer cultures, the cells become grossly elongated and distorted when they adhere to the plastic substratum of the culture flask. The present cells maintain the size and ultrastructure that they possess in the skin. Cells cultured as spherical aggregates form cell junctions (34), possess cell adhesion molecules, and have the ability to synthesize proteins (4) and form cell adhesion and junctions (34) similar to those found in the tissue of origin. In parallel with keratinocytes in skin (28, 37) and fibroblast-populated collagen lattices (7), the fibroblast aggregates used in the present study demonstrated tight junctions, suggesting that cell-cell communication might take place. However, no attempt was made, for example, by using Lucifer yellow, to demonstrate whether this actually was the case. Nevertheless, the demonstration of tight junctions suggests that the fibroblasts still maintained their ability to form cell-cell communication. We therefore believe that these aggregates are a good in vitro model for mimicking the interstitium.

Pressure in the human dermal fibroblast cell aggregates (Pf) was measured with a micropuncture method previously used to measure Pf in vivo in mice, rats, cats, and dogs (24). The only practical consideration when measuring Pf in an aggregate is that it must be anchored to the petri dish, because the pipette could not otherwise be advanced into the aggregate because of aggregate movement. Otherwise, the micropuncture method was used as in previous in vivo studies.

The primary aim of this study was to establish and characterize an in vitro model to study dynamic changes in cell-matrix interactions through the response on Pf as an alternative to using collagen gel contraction assays. The collagen gel contraction assay is based on the ability of fibroblast cells to organize and compact a collagen lattice. Briefly, ~100,000 fibroblasts are mixed with soluble type I collagen. The fibroblast cells contract the collagen fibers to between 10% and 20% of their starting volume within 24 h (14, 33). The contraction is mediated via heterodimeric β1-integrin cell-matrix receptors. The rate of gel contraction is dependent on several factors, including the type and number of cell-matrix attachments as well as the cytoskeleton to which the integrins are attached. Cytokines and chemokines influence the affinity and avidity of the integrins and can therefore exert an effect on the rate of contraction of the collagen gel in the contraction assay. Agents that influence the speed of contraction will either enhance or diminish the rate of contraction, but usually several hours are required to detect a response. The primary aim of the present study was to develop a method with a more rapid response than can be obtained with the collagen gel contraction assay. It was also important that the in vitro model described in this study gives a response to compaction or expansion of the aggregate in opposite directions. In this respect it differs from the collagen gel contraction assay, in which, although there are different rates of response, contraction forces are measured in one direction. The aims of this study were accomplished in that the cell aggregate system responded rapidly (within 1–2 min) and consistently in both positive and negative directions to the pharmacological agents applied. Pf in the control was positive, and the response to PGE2 was in agreement with the in vivo response observed previously (2).

To the best of our knowledge Pf has not been measured previously in human dermal fibroblast cell aggregates, and the observations reported here allow suggestions as to the mechanisms that generate and influence Pf. To more fully explain the model, we also discuss parallel observations in vivo and in other experiments. Under control conditions, Pf in normal fibroblast cell aggregates was ~2 mmHg. The most likely explanation for the positive pressure relative to the ambient fluid in the aggregates would appear to be that water is transported into the central part of the aggregate by active cellular transport and then transported by convection from the interior of the aggregate into the surrounding medium. In addition, there are tensile forces generated by the fibroblast that would also appear to contribute to the normal balance of forces in the normal steady state. These suggestions are founded on the observation that Pf fell and increased in parallel with a lowering or raising temperature, i.e., Pf is dependent on processes requiring energy. Several studies have recently quantified the force produced by single cells (35), although the size of the force measured varied according to the method used. Fibroblast-populated collagen gel lattice (FPCL) studies and the use of force transducers measured a force of 0.1–2 nN per cell (3, 5). Contractile forces, generated by single fibroblast cells with elastomer sheets, demonstrated 2.0 μN per cell (33), an ~1,000-fold higher value than the FPCL studies. Because the FPCL method measures force and stiffness of whole populations, the difference is probably due to this. There is, however, a general agreement that human dermal fibroblasts can generate tension. The α-actin filaments in human dermal fibroblasts can produce force (38). The force generated by actin microfilaments is transmitted from a cell to the extracellular matrix via the integrin receptors (6, 35). As shown in this study, human fibroblast cells are in contact through junc-
tions, thus contributing a cellular network, which is important in the transmission of contact throughout the aggregate. Adhesion properties and expression of extracellular matrix receptors bind the fibroblast cells together as well as to the extracellular matrix, but they differ among different fibroblast populations (23). Integrins constitute a large family of adhesion receptors. The $\beta_1$-integrins have an important role in tension generation in dermal fibroblasts by mediating the force generated from the cytoskeleton through the cell membrane via the integrins and on to the extracellular matrix. This was shown in dermal fibroblasts embedded in the collagen gel, when force generation was effectively inhibited with antibodies that reversibly blocked ligand binding via the $\beta_1$-integrins and the extracellular matrix (10, 14, 15, 26, 29).

The model, which is visualized for the cellular control of $P_f$, is based on in vitro experiments by Meyer (19) as well as our own experiments. Umbilical cord, which is a typical loose connective tissue, will double its volume in 24–48 h when placed in saline. The swelling property is generated by the tissue’s content of hyaluronan and glycosaminoglycans. At the same time, the swelling is restrained by microfibrillar and collagen networks within the tissue because enzymatic degradation of these networks enhances the swelling. Perturbation of $\beta_1$-integrin function with monoclonal antibodies to this receptor, or monoclonal antibodies to the $\alpha$-$\beta_1$-integrin, induces lowering of $P_f$ concomitant with edema formation (26, 27). A series of parallel in vivo and in vitro experiments have demonstrated similar responses in that the agents that reduce the speed of contraction in the collagen gel contraction lattice induce a lowering of $P_f$, whereas agents that enhance the rate of contraction reverse a lowering of $P_f$. In light of these observations and those of Meyer (19), the model that is used to describe the cellular effects in control of $P_f$ is as follows: the perturbation of the cell-matrix contact will reduce the tension mediated from cells on the fiber networks and allow the tissue to swell because of hyaluronan and glycosaminoglycan content. When no fluid is available to enter the tissue initially $P_f$ will fall until a new balance is reached between the lowered $P_{if}$, the ability to swell, and the stress in the filament networks induced by the cellular tension and their inherent biochemical properties. The lowered $P_{if}$ will move water from capillaries and subsequently raise $P_{if}$ toward and above control values again until a new steady state is reached between $P_{if}$, the swelling properties, and the stress in the fiber networks.

The second aim of this study was to elucidate whether the aggregates respond to pharmacological agents previously demonstrated to have an effect on $P_{if}$ in vivo and the collagen gel contraction assay that led to the model described above. Application of PGE$_1$ to the aggregates induced a rapid fall in $P_f$. PGE$_1$ binds to prostanoid receptors of the EP type and prostacyclin receptors (IP) (21), suggesting that the normal tensile forces within the fibroblasts would be reduced by loosening or diminishing their attachments. The lowering of $P_f$ after PGE$_1$ is in parallel to the effect on $P_{if}$ when PGE$_1$ is injected in vivo (2) and a reduction in the collagen gel contraction rate (2). This also corresponds to previous studies using PGE$_1$ and interleukin-1, which showed inhibition of fibroblast-mediated collagen gel contraction (2, 8, 9, 33).

With the application of latanoprost, a PGF$_2\alpha$ analog and selective prostanoid FP receptor agonist, the effect was opposite to that of PGE$_1$. Latanoprost was previously shown to reverse the decrease observed in $P_{if}$ during inflammation, although it had no effect on $P_{if}$ by itself (2). Binding of latanoprost to the FP and $\beta_1$-integrin receptors was probably the reason for contraction of the fibroblasts and thus the enhanced $P_f$.

Because of the effects of PGE$_1$ and PGF$_2\alpha$, prostanoid receptors EP, IP, and FP must be expressed on the human dermal fibroblast cells. The affinity and avidity of the $\beta_1$-integrins in the fibroblast aggregates are also most likely modulated by prostaglandins, allowing for rapid changes in the balance between grip and release.

Ouabain, which is a well-known Na$^+$-K$^+$-ATPase inhibitor, raised $P_f$ in the fibroblast aggregates. The response was in the expected direction because inhibition of Na$^+$-K$^+$-ATPase will cause the individual cell to swell, in turn raising $P_f$. Together, these experiments demonstrate that $P_f$ is influenced by two individual processes: first, the cell-matrix interaction, as demonstrated by the effects of PGE$_1$ and PGF$_2\alpha$; and second, cell volume, as demonstrated by the use of ouabain causing cell swelling and increased $P_f$. The effect of temperature is likely due to a combination of two processes, a diminished contraction of the cells in the aggregates on the matrix (31) and cell swelling caused by diminished pumping activity of the Na$^+$-K$^+$-ATPase (13, 16). Both the Na$^+$-K$^+$-ATPase- and the cytoskeleton-induced compaction on the matrix are energy-dependent processes. This effect is similar to ouabain with reduced pumping activity because the Na$^+$-K$^+$-ATPase is an energy-dependent process. Energy-dependent processes in general are reduced by 50% for every 10°C lowering of temperature. However, the overall effect of cooling was opposite to that of ouabain, and therefore other processes must also be involved. The effect of PGE$_1$ is to diminish the cell matrix, which is in parallel with the observation that lowering the temperature will lower the cytoskeleton-induced contraction in collagen gels (22, 30) and in isolated neonatal rat myocardial cells (30). The observations that the two energy-requiring processes of Na$^+$-K$^+$-ATPase- and cytoskeleton-induced contraction affect $P_f$ in opposite directions, combined with the fact that the overall effect of lowering temperature decreases $P_f$, strongly suggest that cytoskeletal relaxation is overriding the effect of Na$^+$-K$^+$-ATPase on $P_f$.

Although the pressure in the aggregates is positive, the responses to pharmacological agents are in a direction and of a magnitude similar to those observed in vivo. Although the response is more short-lasting than in vivo (about half that seen in vivo), it is nevertheless of a magnitude similar to that seen in vivo. This is
possibly caused by the excess of free fluid surrounding the aggregates (“washing” out the pharmacological agents quickly), which is not the case for skin and dermis in vivo.

Thus the aggregates respond in a manner that is in parallel to the in vivo situation as well as the collagen gel contraction assay. This would then confirm the model for control of interstitial fluid pressure developed based on these systems. The spheroids have a time response and magnitude and direction of the pressure response very similar to those seen in vivo. This will allow for rapid screening of substances for in vivo use, as well as for an improved understanding of which connective tissue cells participate in control of interstitial pressure.

In conclusion, we have developed cellular aggregates that can be used to study dynamic interactions in the connective tissue and are an effective alternative to the collagen gel contraction assay. Cytoskeletal contraction, cell volume regulation, and cell-matrix interactions influence P*r as demonstrated in the present experiments. A positive P*r was observed with increasing size, age, measured depth, and temperature. The human dermal fibroblast cell aggregates provide rapid responses (within minutes) in a more positive or negative direction. Finally, the present study provides evidence for the active modulation of fluid pressure via fibroblasts in the connective tissue.

DISCLOSURES

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