Swelling and pressure-volume relationships in the dermis measured by osmotic-stress technique

Maria P. McGee, Michael Morykwas, Nicole Levi-Polyachenko, and Louis Argenta

Swelling and pressure-volume relationships in the dermis measured by osmotic-stress technique. Am J Physiol Regul Integr Comp Physiol 296: R1907–R1913, 2009. First published March 25, 2009; doi:10.1152/ajpregu.90777.2008.—Water transfer across the extracellular matrix (ECM) involves interstitial osmotic forces in as yet unclear ways. In particular, the traditional values of Starling forces cannot adequately explain fluid transfer rates. Here, we reassess these forces by analyzing fluid transfer in live pig and human dermal explants. Pressure potentials were controlled with inert polymers adjusted by membrane osmometry (range = 3–219 mmHg), and fluid transfer in and out of the explants was followed by sequential precision weighing. Water motional freedom in the dermis was examined by NMR. In pigs, mean hydration pressure (HP; the pressure at which volume did not change) was 107 ± 22 and 47 ± 12 (SE) mmHg at 4°C and 37°C (P = 0.012, paired t-test, n = 7). Volume changes observed in response to pressure potential were reversible. The equation, Volume change = \( V_{\text{max}} / [1 + (t/T_{1/2})^{a}] \), where \( V_{\text{max}} \) is maximal volume change; \( T_{1/2} \), time at volume = 1/2 \( V_{\text{max}} \); and \( a \), a rate parameter, was fitted to experimental progression curves (\( r^2 > 0.9 \)), yielding \( V_{\text{max}} \) values linearly related to pressure, with mean slopes \(-3.5 \pm 0.28 \) and \(-2.6 \pm 0.21(\text{SE}) \mu l/g^{-1} \text{mmHg}^{-1} \) at 4°C and 37°C. NMR spin-spin relaxation times (\( T_2 \)) varied within 200- to 400-μm distances in directions perpendicular to the epidermis, with slopes reaching 0.03 ms/μm. Results support a mechanism in which fluid transport across the ECM is locally regulated at micrometer scales by cell- and fiber-gel-dependent osmomechanical forces. The large HP helps to explain the fast interstitial in/out flow rates observed clinically.

Address for reprint requests and other correspondence: M. P. McGee, Plastic and Reconstructive Surgery Research, Surgery Division, Wake-Forest Univ. Medical School, Medical Center Blvd., Winston-Salem, NC 27157 (e-mail: mmcgee@wfuwbnc.edu).

http://www.ajpregu.org 0363-6119/09 $8.00 Copyright © 2009 the American Physiological Society R1907

Swelling and pressure-volume relationships in the dermis measured by osmotic-stress technique

Maria P. McGee, Michael Morykwas, Nicole Levi-Polyachenko, and Louis Argenta

Swelling and pressure-volume relationships in the dermis measured by osmotic-stress technique. Am J Physiol Regul Integr Comp Physiol 296: R1907–R1913, 2009. First published March 25, 2009; doi:10.1152/ajpregu.90777.2008.—Water transfer across the extracellular matrix (ECM) involves interstitial osmotic forces in as yet unclear ways. In particular, the traditional values of Starling forces cannot adequately explain fluid transfer rates. Here, we reassess these forces by analyzing fluid transfer in live pig and human dermal explants. Pressure potentials were controlled with inert polymers adjusted by membrane osmometry (range = 3–219 mmHg), and fluid transfer in and out of the explants was followed by sequential precision weighing. Water motional freedom in the dermis was examined by NMR. In pigs, mean hydration pressure (HP; the pressure at which volume did not change) was 107 ± 22 and 47 ± 12 (SE) mmHg at 4°C and 37°C (P = 0.012, paired t-test, n = 7). Volume changes observed in response to pressure potential were reversible. The equation, Volume change = \( V_{\text{max}} / [1 + (t/T_{1/2})^{a}] \), where \( V_{\text{max}} \) is maximal volume change; \( T_{1/2} \), time at volume = 1/2 \( V_{\text{max}} \); and \( a \), a rate parameter, was fitted to experimental progression curves (\( r^2 > 0.9 \)), yielding \( V_{\text{max}} \) values linearly related to pressure, with mean slopes \(-3.5 \pm 0.28 \) and \(-2.6 \pm 0.21(\text{SE}) \mu l/g^{-1} \text{mmHg}^{-1} \) at 4°C and 37°C. NMR spin-spin relaxation times (\( T_2 \)) varied within 200- to 400-μm distances in directions perpendicular to the epidermis, with slopes reaching 0.03 ms/μm. Results support a mechanism in which fluid transport across the ECM is locally regulated at micrometer scales by cell- and fiber-gel-dependent osmomechanical forces. The large HP helps to explain the fast interstitial in/out flow rates observed clinically.

THE LOCAL MECHANISMS THAT balance interstitial fluid volume and prevent edema, swelling, and dehydroxylation in the skin are not sufficiently understood. The role and relative importance of intrinsic factors other than transcapillary pressures and vascular permeability changes remain particularly uncertain (1, 2, 3, 4, 11, 17).

The skin interstitium contributes significantly to systemic fluid homeostasis. The dermal interstitial matrix is estimated to hold 2–3 times as much fluid as circulating blood, exchanging 3–6 liters per day, at least one-third drained by the lymphatic circulation (11, 26, 34). Fluid is filtered via the semipermeable walls of capillary beds and reabsorbed via the walls of post-capillary venules and lymphatic capillaries (34). According to the Starling hypothesis (25, 1, 2), the driving pressure for this process is the net result of colloidosmotic and hydrostatic pressures in the capillaries and interstitium. However, in the vascular network, the pressure drop from precapillary to post-capillary areas is only a few millimeters of mercury, although it can fluctuate widely—to ~20 mmHg—with capillary perfusion and tone (11, 33). The interstitial pressure components are much less accessible to measurement, and reported values can vary ~10 mmHg above and below atmospheric pressure (11, 3). Moreover, the rate of fluid reabsorption in hypovolemic shock and edema formation in burns seems to exceed limits predicted by the Starling principle (7, 10, 12); pressure deflections (~100 mmHg below atmospheric pressure) measured in excised, heat-treated dermis and clinically effective, subatmospheric pressures in vacuum-assisted wound healing (generally in the 75–125 mmHg range) are much larger than reported for interstitial pressures. These inconsistencies have not been satisfactorily explained and call for new fluid balance models that accurately measure the interstitial matrix’s contribution.

Interstitial swelling pressures have been measured in vivo and in vitro using a variety of techniques but not as accurately as their intravascular counterparts (3, 8, 15, 16, 18), yielding inconclusive information on the matrix’s contribution to fluid transfer (2, 5, 11). More quantitative data on the nature and relative magnitude of swelling forces (3, 9, 15, 16, 18, 19) are needed to explain experimental and clinical observations, as well as to develop and evaluate new therapeutic approaches to decrease or to prevent edema (7, 12, 30, 31).

Here, we adapt osmotic stress techniques (13, 14, 20, 21, 24), pioneered to study macromolecular interactions in aqueous solutions, to control water potentials and to measure fluid transfer kinetics to and from metabolically active dermal explants. Kinetic studies are complemented by high-resolution NMR water proton transverse relaxation time (\( T_2 \)) determinations, exploring water motional freedom at resolutions in the 100 μm–mm range in situ. From the results, we extract new quantitative information, indicating surprisingly large interstitial pressures, consistent with the existence of local fluid transfer balance mechanisms.

MATERIALS AND METHODS

Dermal explant preparation. Full-thickness samples were excised from pig dorsal and human ventral skin. Approximately 10 × 4 cm strips were carefully trimmed from fat, fascia, and muscle layers, avoiding air exposure by keeping them wrapped in paraffin paper during processing.

Pig and human skin samples were obtained as by-products from unrelated animal studies and elective abdominoplasties, respectively. Typically, pig skin was collected immediately after euthanasia, while human skin was collected immediately after being excised during surgery. Explants were prepared by cutting replicated pieces of approximately equal size, 0.5 × 0.5 × 0.3 mm each, and placing them in culture medium (DMEM/F12, Invitrogen, diluted to 1/2 strength) containing 5% FCS, the antibiotics streptomycin and penicillin, and 12 mM HEPES.

Address for reprint requests and other correspondence: M. P. McGee, Plastic and Reconstructive Surgery Research, Surgery Division, Wake-Forest Univ. Medical School, Medical Center Blvd., Winston-Salem, NC 27157 (e-mail: mmcgee@wfuwbmc.edu).
buffer pH 7.2–7. The osmotic pressure generated by the crystalloid components of the incubating solution is ~5,800 mmHg at 37°C.

After trimming the fat, we found that the thickness of the explants varied from donor to donor but was generally between 2 and 4 mm. The protocol for obtaining human tissue was approved by the Institutional Review Board before the consent forms were signed by the donating patients. Animal protocols were approved by the Institutional Animal Care and Use Committee. The water content of skin samples was estimated from dry/wet weight ratios. Samples were dried under vacuum at 42°C for at least 20 h. The ratio between dry and wet weight was 0.372 (0.064) mean (SD), ranging from 0.264 to 0.455 (n = 15) for pig skin, and 0.370 (0.066) mean (SD), ranging from 0.300 to 0.488, n = 6, for human skin.

Histology. Representative skin explants were examined by light microscopy both in fixed, paraffin-embedded, 6-μm-thick sections stained with hematoxylin and eosin (H&E) and in 40-μm-thick cryostat sections stained with Alcian Blue to identify glycosaminoglycans. Briefly, freshly cut cryostat sections from dermal explants were rinsed with 0.15 N NaCl and incubated in a filtered solution of Alcian Blue (0.5% 0.15 N NaCl, 50 mM MgCl₂, pH 6) at 23°C for 15 min. After washing and staining, the sections were washed with 0.15 N NaCl and incubated in a filtered solution of Alcian Blue at pH 6 for 24 h. The sections were then rinsed with PBS, pH 7.3, and examined under light microscopy.

Fluid transfer measurement and osmotic stress technique. The volume transferred between dermal interstitium and bulk bathing fluids was measured as a function of time by precision weighing of explants. Initial weights were taken in random sets of samples before segregating them into various experimental conditions; subsequent weight measurements were made in each set at a predetermined time sequence. Differences among replicate weight measurements in one given explant were <2% of the mean and in different dermal explants from the same animal <15%. The driving pressure for fluid transfer was controlled to between 3 and 219 mmHg by adjusting colloidosmotic pressure in bulk with inert, neutral polymers, including polyethylene glycol and dextran of 8,000 and 10,000 nominal molecular weight, respectively. Bulk volumes in cultures were at least 10-fold greater than explant volumes to ensure negligible changes in the bulk’s colloidosmotic pressure from tissue-fluid transfer. Colloidosmotic pressures of bulk solutions were measured by reference to standard curves constructed from triplicate measurements of polymer solutions using a membrane osmometer (molecular weight cutoff = 5,000, Wescor 4420) calibrated with a 5% solution of human albumin set at 20 mmHg. Changes in the colloidosmotic pressure of the incubating solution resulting from adding the polymers ranged from 0.5 to 3.7% of the total osmotic pressure; i.e., 35 to 219 mmHg, respectively. They are within the physiological differences in osmotic pressure reported for human sera.

The possibility that significant amounts of the stressing polymer penetrated the matrix during the swelling experiments was excluded by control experiments, in which the magnitude of diffusional transfer into the dermis was explored. Fluorescein-labeled PEG and dextran tracers were used to create 2.5–0 mg/ml concentration gradients across the dermal matrix under isometric, 300 mOsm, isobaric 1 atm, and isothermal 25°C conditions. In dermal explants with or without epidermal layers (with the upper ~200-μm layer removed with a dermatome prior to full-thickness skin excision), less than 3% of tracer was transferred across the matrix in 8 h. Thus, dermal-matrix penetration by the stressing polymers does not contribute significantly to the dissipation of pressure gradients between bulk solution and the matrix during measurement of fluid transfer kinetics.

Control experiments were also conducted to explore possible electrostatic screening or an osmotic contribution from the salts in bulk solution during the swelling process. Increasing the osmotic pressure of bulk solution up to ~28 atm (with 0.6 M NaCl) did not increase initial swelling rates, indicating that electrostatic forces or Donnan-type osmotic effects are not likely to influence the fluid transfer measured.

The viability of dermal explants was confirmed in representative samples using intravital microscopy and the fluorescent nuclear dyes bisbenzimide and ethidium bromide (22). Most cells in the interstitial matrix remained viable, that is, excluded ethidium bromide, even after the longest incubation time examined, ~60 h.

Skin compression measurements. Uniaxial compression tests were conducted in folds of full-thickness skin explants. A constant pressure of 10 g/mm² was applied with spring-loaded levers to a 30 mm² area. The tissue did not exude water during the compression experiments. Its thickness was measured within 0.05 min and at 1- to 20-min intervals for an hour. Measurements were performed sequentially in duplicate after equilibrating skin at either 4°C or 37°C for at least 3 h. For each trial set, pressure was applied at the same site of the skin-fold surface.

Magnetic resonance examination. Water proton transverse relaxation times (T₂) across full-thickness, dermal explants were calculated from a multislice, multiecho sequence following a Carr-Purcell-Meiboom-Gill protocol (6) in a 7-T small animal scanner (Bruker Biospin, Ettlingen, Germany) with a shim insert, producing a gradient of 400 mT/m. Skin (~5 × 3 cm strip) was pre-equilibrated for ~5 h in culture medium at 104 mmHg, then transferred to, and immobilized in, a sealed cylindrical tube during the procedure.

Kinetic and statistical analyses. A nonlinear progression model with time as a power term was used to fit the fluid-transfer kinetics in and out of the dermal explants. Equation selection, curve fitting, descriptive statistics, and paired comparison t-tests were executed using the commercially available software packages TableCurve and StatView (SAS Institute, Cary, NC). In all of the experiments presented here, a P value <0.05 was considered significant. Summarized data from experiments with dermal samples from different subjects are reported as means (SD) or ± SE.

RESULTS

Histologic examination. The general pattern of glycosaminoglycan distribution in the dermis was examined in freshly cut, 40-μm-thick cryostat sections of representative dermal samples stained with the cationic dye Alcian Blue. The epidermis remained unstained, while the dermis stained blue with an intensity increasing in a direction perpendicular to the epidermal plane from the deep to the more superficial dermal layers. Localized differences in color intensity were also apparent in the parallel direction at separations of ~100 μm (i.e., similar to the thickness of the epidermal layer). Figure 1 shows typical results. A representative hematoxylin-and-eosin-stained, 5-μm-thick section is also shown to illustrate the collagen bundles, cell distribution, and other dermal elements not revealed clearly in the cryostat sections. However, the H&E stain procedure includes dehydration-rehydration and paraffin embedding steps that destroy the hydrated glycosaminoglycan structures and distort the tissue architecture relative to that in fully hydrated fresh sections.

Swelling of dermal explants: time dependency. Net fluid transfer into dermal explants incubated in culture media (with 5% FCS generating a colloid-osmotic pressure of 3 mmHg) was measured as a function of time by precision sequential weighing. Volume change, V, was derived from the weight difference between explants at time = 0 and after incubation times ranging from 30 to 1,500 min and expressed as a fraction of the weight, W, at t = 0; V = [W(t) − W(0)]/W(0). The weight of explants increased for ~24 h, after which it changed little for the duration of the experiments, up to 50–60 h (Fig. 2). The rate of change of V decreased with time according to kinetics that are well described by a nonlinear asymmetric transition
curve: Volume change = $V_{max}/[1+(time/T_{1/2})^d]$, where $V_{max}$ is maximal volume change; $T_{1/2}$, time when the volume change is 1/2 of $V_{max}$; and $d$ is a parameter proportional to the rate. Coefficients of determination $r^2$ from the model-fitting data points for each subject were generally >0.98 and standard errors of $V_{max}$ <15%. However, the values of $V_{max}$ and $T_{1/2}$ varied widely among explants from different subjects.

Swelling of dermal explants: reversibility. The reversibility of the swelling response was tested by maintaining the pressure of the culture solution at 3 mmHg for 24 h, then changing it to 135 mmHg for another 24 h and back to 3 mmHg for an additional 24 h. Results demonstrate reversibility, with $V_{max}$ values of 0.435 ± 0.097, -0.423 ± 0.044, and 0.394 ± 0.032 at 4°C and 0.152 ± 0.008, 0.413 ± 0.023, and 0.298 ± 0.007 (SE) at 37°C for the initial swelling, deswelling, and second swelling, respectively; the corresponding $d$ values were -0.759 ± 0.084, 0.550 ± 0.045, and -0.644 ± 0.087 (SE) at 4°C, and -0.842 ± 0.082, 0.667 ± 0.041, and -0.755 ± 0.026 (SE) at 37°C, respectively; the rates at $t = 1$ min were 0.0029, -0.0066, and 0.0029 min⁻¹ at 4°C and 0.0024, -0.0056, and 0.0028 min⁻¹ at 37°C, respectively. Representative results are illustrated in Fig. 3.

Hydration pressures of healthy dermal interstitium. The rather large variation in the ratio between dry and wet weights and in swelling rates observed among the dermal samples obtained from young healthy pigs suggested that physiological hydration pressures in dermal interstitium also vary widely. To explore this possibility, the pressure of explants at isolation was determined by osmotic stress techniques. For these experiments, sets of samples replicated from each individual were incubated with physiological solutions at six levels of colloid-osmotic pressure, ranging between 3 and 219 mmHg. The weight of explants increased (swelled), decreased (shrank), or remained unchanged depending on the colloid-osmotic pressure in the bulk solution (Figs. 2 and 3).
The values of $V_{\text{max}}$, derived for each pressure by fitting the model equation to the experimental progression curves, were linearly related to the colloidosmotic pressure of bulk fluid (Fig. 4). The slope of $V_{\text{max}}$/pressure plots and the hydration pressure (HP) were calculated from fitted regression lines, as summarized in Table 1. HP values expanded from 37 to 160 mmHg colloidosmotic pressure. The $V_{\text{max}}$ values, with HP values determined from first derivatives and the initial rates determined from second-degree polynomials, and the initial rates determined from first derivatives and the initial 120-min interval were fitted with second-degree polynomials. Results for human and pig explants are summarized in Table 1. Subject-to-subject variation was larger than the 10–15% experimental variation expected from explant preparation and multiple weighing. The slopes $V_{\text{max}}$/pressure, and the HP values were correlated with correlation coefficient of 0.837 ($P$ value = 0.0067).

To control for the possible influence of polymer effects other than osmotic pressure, experiments were repeated in two sets of explants from the same animal. Each was tested at six different pressure levels; in one set, the osmotic stress was induced with dextran and in the other with PEG. Results with these two polymers of very different physicochemical structure were similar. Table 2 confirms that the volume changes observed in the dermal explants depend on the colloidosmotic gradients between the bulk fluid and dermis rather than other effects; for example, changes in the osmotic pressure of the polymer solution with pH and temperature (26, 32) or in the viscosity with concentration.

Hydration pressures were also calculated from initial rates of volume change. In these analyses, V/time data points from the initial 120-min interval were fitted with second-degree polynomials, and the initial rates determined from first derivatives at $t = 0$. As with the equilibrium analyses (i.e., using $V_{\text{max}}$/pressure), the initial rates were linearly related to the colloidosmotic pressure of bulk solution with $r^2 > 0.9$ and slope values, $-0.614 \pm 0.161 \times 10^{-5}$ and $-0.946 \pm 189 \times 10^{-5}$ min$^{-1}$ mmHg$^{-1}$ for 4°C and 37°C, respectively, which were significantly different with a $P$ value = 0.0002 in paired t-tests. The HP values were 77 (42) and 39 (45) (SD) mmHg at 4°C and 37°C, respectively, and significantly different in paired t-tests with $P$ value = 0.0001.

Compression of dermal explants: temperature effect. Dermal folds in skin samples from two different pigs and one human were compressed uniaxially by applying a constant pressure of 10 g/mm² perpendicular to the epidermal surface at either 4°C or 37°C. The applied pressure decreased the pig skin thickness to 0.519 (0.026 SD) and 0.544 (0.031) of its uncompressed value at 4°C and 37°C, respectively. Most of the change, 0.351 (0.047 SD) and 0.392 (0.086 SD), respectively, was immediate, within 0.005 min, while changes of 0.169 (0.041 SD) and 0.152 (0.076 SD), respectively, took place over time. The temperatures did not generate statistically significant differences, with $P$ values > 0.225 in paired t-tests with data pairs from four trial sets. The results from measurements of the one human skin sample were similar and are illustrated in Fig. 5.

NMR spin-spin relaxation time. Values for the decay constant ($T_2$) at different depths were calculated in full-thickness explants after incubation for ~5 h in bulk solution at 104 mmHg colloidosmotic pressure. The $T_2$ values varied across the dermis; typical results are illustrated in Fig. 6, one of three similar experiments with samples taken from three different

<table>
<thead>
<tr>
<th>$V_{\text{max}}$/Pressure*, μl/g/mmHg</th>
<th>HP†, mmHg</th>
<th>$\Delta$HP‡, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Pig</td>
<td>Human</td>
</tr>
<tr>
<td>4°C</td>
<td>-2.9±0.8</td>
<td>149±18</td>
</tr>
<tr>
<td>37°C</td>
<td>-1.8±0.3</td>
<td>74±17</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE for humans (n = 4) and pigs (n = 7) of slopes determined from $V_{\text{max}}$ vs. pressure plots by linear regression analyses; values of regression coefficients were $>0.83$. Volume changes were measured by precision weighing during equilibration of full-thickness skin explants in solutions adjusted to colloidosmotic pressures ranging from 3 and 219 (0–12% wt/wt polyethylene glycol) mmHg, incubated at either 4 or 37°C. The $P$ values indicated the difference between the HP determined at 4 and 37°C in paired t-tests. *$V_{\text{max}}$ = maximal volume change calculated from progression curves fitted with equation: $V = V_{\text{max}}/[1 + (\text{time} / T_1/2)^2]$ for each pressure increment. †HP = hydration pressure or pressure at which explant’s volume did not change. Values are calculated from the linear regression curve at $V$ = 0. ‡$\Delta$HP = difference between the hydration pressures determined at 4°C and 37°C.

Table 2. Volume-pressure relationships in human and pig dermis explants determined by osmotic stress: comparison between responses at 4 and 37°C

Table 2. Volume-pressure relationships in porcine skin determined by osmotic stress induced with inert, excluded polymers: Comparison between Dextran 10T (DT) and polyethylene glycol 8000 at 4 and 37°C

<table>
<thead>
<tr>
<th>$V_{\text{max}}$/Pressure*, μl/g·mmHg·1·mmHg$^{-1}$</th>
<th>HP*, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>PEG</td>
</tr>
<tr>
<td>4°C</td>
<td>90</td>
</tr>
<tr>
<td>37°C</td>
<td>44</td>
</tr>
</tbody>
</table>

Values are the slope ± SE determined from $V_{\text{max}}$ vs. pressure plots by linear regression analyses; regression coefficients are in parenthesis. Volume changes were measured by precision weighing during equilibration of full-thickness skin explants in solutions adjusted to colloidosmotic pressures ranging from 3 to 115 [0–12% wt/wt dextran 10T (DT)] and 3 to 135 [0–6% wt/wt polyethylene glycol 8000 (PEG)] mmHg. All 24 explants used for these measurements were prepared with skin from the same animal. *$V_{\text{max}}$ = maximal volume change calculated from fits of equation: $V = V_{\text{max}}/[1 + (\text{time} / T_1/2)^2]$ to progression curves for each pressure increment. †HP = hydration pressure, or pressure at which an explant’s volume did not change. Values are calculated from the linear regression curve at $V$ = 0.

Fig. 4. Volume-change/pressure relationships in dermal explants. The $V_{\text{max}}$ values are plotted as a function of the pressure. The slope was $\mu$l·g$^{-1}$·mmHg$^{-1}$. Each $V_{\text{max}}$ data point was determined by fitting the model equation to each experimental progression curve in Fig. 2. The hydration pressure value is the pressure at which the rate does not significantly differ from 0; i.e., the pressure in the matrix matches that in the medium, and the potential for fluid transfer is 0 (the intersection of the curve and the horizontal dotted line in the figure). Table 2 shows the mean and standard deviations of hydration pressures determined in different subjects (7 pigs and 4 humans).
The kinetic studies revealed interesting characteristics of the local control of the dermal fluid transfer process. Fluid transfer to and from the interstitium follows a relatively simple progression curve that can be fitted by a transition function, with time in a power term. When dermal explants are placed in medium with a colloidosmotic pressure different from their initial hydration pressure, their volume changes, rapidly at first, but then slowly, approaching a maximum. The equation fitted to the time course of dermal volume changes yielded parameters useful for comparing responses among samples with different initial hydration levels. Particularly, the $V_{\text{max}}$ parameter varied linearly with the pressure gradient both in human and pig explants, giving slopes that were temperature dependent. From differences in volume/pressure at 4°C and 37°C, the temperature-dependent component of the interstitial pressure is estimated at $\sim 70$ mmHg and similar in human and pig dermis. These findings suggest local dermal regulatory mechanisms that include energy-dependent processes in addition to the familiar “passive” responses to transcapillary colloidosmotic pressure differences (2, 3, 25). They also support and add key quantitative information to more recent hypotheses that postulate an active role for connective tissue fibroblasts in fluid homeostasis (23, 30).

Among other explanations that do not exclude cell action is the possibility that temperature and pH shifts change the physicochemical properties and mechanical characteristics of the fiber mesh by processes other than interference with the enzyme-dependent, energy-generating pathways. The fact that a 4°C to 37°C temperature difference does not appear to influence skin responses to a constant uniaxial load is consistent with the idea that temperature-dependent differences observed in the colloidosmotic stress experiments likely reflect metabolic factors in addition to viscosity and/or macroscopic elasticity changes with temperature. On the other hand, the two different stressing methods used are unlikely to probe temperature effects on skin viscoelastic properties in comparable ways, particularly time dependence. The structure and function of the live interstitium are so completely integrated that attempts to separate effects on material properties from effects that depend on metabolically derived energy are perhaps futile within physiological temperature ranges.

The fibroblasts in the dermal interstitial matrix are mobile and enmeshed in a glycosaminoglycan gel that is not evenly distributed across the dermis, judging from the intensity of the Alcian Blue dye (Fig. 1B). Because the osmotic properties of
glycosaminoglycans depend on their local concentration, their apparent heterogeneous and anisotropic distribution under light microscopy suggests that osmotic gradients can develop in the dermal layers at these micrometer scales. The NMR spin-spin relaxation results in live explants are consistent with the existence of water-potential gradients at micrometer-millimeter scales; the $T_2$ parameter measured at points perpendicular to the skin surface varied with the distance from the epidermis. This parameter reflects the relative motional freedom of local water molecules, as modified by interactions with neighboring molecules; spin-spin relaxation times are determined by the degree of interaction, and therefore, space-dependent differences in their value reflect corresponding local differences in water activity (relative to pure water). Although the local differences observed in the $T_2$ parameter do not identify the specific water interactions, they are also consistent with a dynamically regulated model. We hypothesize that local contraction/relaxation of the fiber-gel mesh controlled by the cells could also contribute to maintain local differences in water potential, which, in turn, would lead to transitory pressure gradients and directional flows at scales determined by the cells’ region of influence.

The present studies probed biologically relevant material properties of the dermis. Its responses to pressure and temperature changes suggest an expanded version of a model first proposed by Meyer and colleagues (15, 16), in which glycosaminoglycan swelling was simply balanced by the mechanical tension developed in the fiber mesh. Globally, the quantitative data presented here, together with information in the literature, add fibroblast action to the glycosaminoglycans-fiber osmomechanical forces (23, 29, 30, 31) and point toward rich and complex modulatory responses to osmotic gradients, mechanical tension, and oxygen by the interstitial matrix. The local nature of this cell-dependent regulatory component calls for further theoretical and experimental studies to identify the cell-gel-fibron collagenous unit both histologically and mechanistically.

**Perspectives and Significance**

The variation in hydration pressures found in dermal explants from young animals with ostensibly healthy skin and confirmed in humans implies the existence of local factors capable of maintaining interstitial fluid volume within physiological ranges. Cell-controlled pressures of this order of magnitude could mediate compensatory reabsorption during hypovolemic shock by rapidly redirecting interstitial volumes. They would also help to explain the fast rate at which dermal edema fluid accumulates with the cell injury secondary to burns (7, 10, 12, 19). Together, with previous findings on macromolecular reaction kinetics (13, 14, 21), these results point to future work based on the hypothesis that water potentials in biological media integrate transfer mechanisms across temporal and spatial scales.

**ACKNOWLEDGMENTS**

We thank Denis Grebenkov for helpful discussion on the model equation parameters and Institute for Pure and Applied Mathematics at UCLA for enabling them. Lynne Li is recognized for her meticulous technique and valuable assistance with data reduction.

**REFERENCES**


