Modeling in vivo interstitial hydration-pressure relationships in skin and skeletal muscle

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ABSTRACT

A theoretical understanding of hydrostatic pressure- fluid volume relationships, or equations of state, of interstitial fluid in skin and skeletal muscle through mathematical/physical modeling is lacking. Here we investigate at the microscopic level forces that seem to underlie and determine the movements of fluid and solid tissue elements on the microscopic as well as on the macroscopic level. Effects that occur during variation of hydration due to interaction between expanding glycosaminoglycans (GAGs) and the collagen interstitial matrix of tissue seem to be of major importance. We focus on these interactions that let effects from spherical GAGs that expand and contract relative to collagen on microscopic level as hydration changes and generate a hydration dependent electrostatic pressure on the extracellular matrix on the microscopic level that spreads to macroscopic levels and become a key factor for setting up equations of state for skin- and skeletal muscle interstitia. The modeling for a combined skeletal muscle- and skin tissue is one-dimensional, i.e. a flat box that may mimic central transverse parts of tissue with more complex geometry. Incorporating values of GAG and collagen densities and fluid contents of skin- and muscle tissues that are of an order of magnitude found in literature into the model gives interstitial hydrostatic pressure- fluid volume relationships for these tissues that agree well with experimental results.

List of symbols

ECM, extracellular matrix; ECM+ cell, extended ECM cell, including cell fluid; GAG, glycosaminoglycan; α, number $\pi/6$.

Subscrips and bars: 1, inside of GAG; 2, outside of GAG; A, atmospheric; c, cell; col, collagen;D, Darcy; E, electrostatic; F, force; f, fluid; f,Sph, interaction spherical force; i, interstitial; if; interstitial fluid; Q, due to electric charge Q; s, solid; v, vascular; y, in y direction; int, interaction; col, collagen, $\overline{h}$, average of *h* over right half thickness of GAG-box in sphere-box model; $\tilde{h}$, average of *h* over ECM cell.

Variables: A, Area= z^2; M, mass; n, steepness parameter force response; Q, GAG charge; R, spherical GAG radius; V, macroscopic volume; *v*, either microscopic volume (as v_{cvdry}, microscopic volume of cell and vascular dry material), or velocity (v_{if}, interstitial fluid velocity); $X=\alpha R$, corresponding half GAG thickness in sphere box model; y, half thickness of ECM-cells on nanometer or micrometer scale, also measure of hydration; y_0, smallest value of y; $y*$, largest value of y; z, sides of quadrate transverse thickness ECM-cell; ξ, position along thickness of ECM-cells; ξ_0, position variable on microscopic ECM-scale; ξ_1, position variable on macroscopic (tissue) scale (mm or cm), W1, experimental hydration (ml interstitial fluid/g dry weight); W2, experimental hydration (ml interstitial fluid/g wet weight); w1, model hydration corresponding to W1; w2, model hydration corresponding to W2.

Physical constants, coefficients, parameters: *a*, collagen fractional volume; f_s, collagen fraction of y_0; f_c, multiplicative factor, cell fluid in ECM+ cell relative to interstitial fluid volume part; ε_0, vacuum permittivity; κ_1, κ_2, dielectric constants inside, outside GAG; κ_e, elastic modulus; $k_{12}=\kappa_1/\kappa_2$; s_0, a tensile related force constant

Fields, forces, force-related expressions and functions:

F_Q, summed up electrostatic force on GAG within a spherical GAG unit surface element; F_{int}, summed up GAG-collagen interaction force within unit surface spherical GAG element; H, unit step function; p, pressure ; S, a tensile force; u, compressions and elongations of collagen in y-directions; u_m, u-value at $y*$; v_{if}, velocity (of interstitial fluid); $b=(k_{f,Sph}(y*)/k_{f,Sph}(y_0))^{1/5}=R(y_0)/R(y*)$, a GAG radii ratio; ρ, specific weight; $k_{f,Sph}(y)$, GAG-collagen interaction force density within spherical GAG at hydration y; k_Q, parameter electric forces; $KQkf=(k_Q/k_{f,Sph}(y*))^{1/5}$, GAG radius parameter; $f_{y,\text{int}}=k_{f,Sph}(y)/2$, smeared out force density in the y-direction of GAG half-sphere; $\overline{f_{y,\text{int}}}$, smeared out GAG-collagen interaction force density in thickness direction of a corresponding box shaped GAG.

INTRODUCTION

Interstitial compliance, defined as the change in interstitial fluid volume, ΔV_{if} divided by the corresponding change in interstitial fluid pressure, Δp_{if}, is of critical importance for interstitial fluid volume regulation because this parameter determines the hydrostatic counterpressure to a given change in V_{if}. Thereby, in a tissue with low compliance, increased net filtration, resulting from, for instance, increased capillary pressure, will be counteracted by a marked increase in p_{if}, for a modest rise in V_{if}. Conversely, a tissue with high compliance will allow considerable rise in V_{if} before p_{if} rises. In the two organs containing most of the interstitial fluid volume, skin and muscle, the p_{if} is slightly subatmospheric in control situation. p_{if} falls abruptly upon dehydration, whereas an overhydration will produce an initial rise in p_{if} that upon further rise in V_{if} stabilizes at a level slightly above atmospheric pressure (1) before rising abruptly again upon extreme overhydration (2). Interestingly, a lower than normal fixed electric charge of the tissue results in a p_{if} that is less negative, i.e. higher than in normal states (1, 3). The compliance phenomenon will through p_{if} be a determinant of bulk flow of fluid into tissues, including e.g. uptake of macromolecular agents like monoclonal antibodies in tumors.

A theoretical explanation of the volume-pressure relationships in soft tissues have up till now relied on work by Meyer (4), assuming an equilibrium between oncotic pressure of a hyaluronic acid solution combined with experimental data of swelling pressure in umbilical cord. In the present paper, we aim at providing a full theoretical model explaining the volume-pressure relationship in skin and muscle where experimental data are available that can be extrapolated to tissues like tumors where such data are unavailable. Our modeling is based on original recordings of interstitial fluid volume obtained by in vivo distribution of an extracellular (^{51}Cr-EDTA) and an intravascular tracer (tagged erythrocytes or ^{125}I-albumin) that is a reference method to assess such volumes, and weighing of tissue samples. These data have been translated into hydration in the model. This was possible because the samples were excised from the experimental animals. Such excision makes it possible to do an accurate determination, but has the disadvantage that the method is invasive. There are of course non-invasive methods like MRI and bioimpedance that are used clinically that will be able to reflect hydration in some way, but not with the same resolution as tracer methods. It might be possible to translate e.g. MRI data to hydration, but it is not a trivial exercise (if possible) to assess interstitial fluid volume with this method. Still, our data might be useful in future translational studies in this area.

It should be observed that skin in the present context is the same notation as used in the experimental studies forming the basis of the model, although the experimental volume and pressure data are mostly derived from dermis (5) that from a fluid balance perspective is most interesting, and not from the underlying

subcutaneous fat. In the model, we will consider interactions between major elements of the extracellular matrix (ECM), i.e. elastic collagen and glycosaminoglycans (GAGs) with their fixed charges and interstitial fluid at varying degrees of hydration.

We hypothesize that the observed shape of interstitial fluid volume-pressure relationship is influenced by hydration dependent electrostatic pressure due to GAG fixed charges: This pressure originates at the microscopic level on nano-to-micrometer scale in the interstitium where GAG charges interact with the collagen matrix with a strength that depends on tissue hydration. Thereby matrix expansion on both microscopic and macroscopic levels will also be dependent on the degree of hydration. In modeling of cartilage and expansive tissue forces, effects of hydration in the interstitium seems of less or no importance, and electrostatic pressure in such tissues has its origin on interaction between rather compactly packed GAGs on the nanometer (nm) or micrometer (mm) scale via anions and cations in the tissue (6, 7). Effects of anions and cations are incorporated in our modeling, but is limited to the effect they have to counteract fixed charges self-expansiveness. This self-expansive property of fixed charges and the way it takes place as hydration changes, and the associated expansive electrostatic pressure on the collagen matrix via the GAG-collagen interaction force, leads to the relation between hydrostatic pressure and interstitial hydration. Our model may thereby also provide new insight into the quantitative role of negatively charged GAGs in relation to storage of Na^+ in skin that has recently been shown to have a buffer role in salt-sensitive hypertension(8, 9).

The present model study highlights only those mechanisms that seem to be most important and necessary to explain the interstitium equations of state: Forces within the interstitium are limited to electrostatic forces, hydration-dependent interaction forces, hydrostatic forces and elastic- and tensile forces. Forces on the collagen matrix due to fluid flow through the interstitium have been left out because in comparable in vivo experiments these forces can be nulled out. In the present modeling of skin and muscle interstitium it is important to incorporate differences in volumes of cell fluid and solid material in addition to collagen of the ECM. Thus the extracellular matrix-cells (ECM cells), the ”building blocks” on microscopic level introduced in a previous paper (10), is extended in the present model to ECM+ cells where also ordinary cell fluid volume and cell- and vascular solid masses are included. This extension of building blocks facilitates the comparison between model and experimental results. The model can be extended to malignant tissues by varying parameters introduced below, and thus open up for modeling convection and diffusion of macromolecules and thereby monoclonal antibodies used therapeutically through such tissues at varying degrees of hydration.

METHODS

General outline of the model

We recently modeled skin tissue on the microscopic level as composed of specific extracellular matrix cells (ECM cells) (10) on nano- to micrometer scale, and were able to predict data in complete agreement with those from in vitro volume exclusion experiments (11, 12). In particular, two models for ECM cells were developed: A simple box model, and a sphere-box model. A collagen network spans over each ECM cell, and in the central regions in both models, GAGs with their fixed negative electric charges are situated. In the simple box model, GAGs are box shaped

and reach out to distances x and $-x$ within each ECM cell, which reaches out to y and $-y$, $|y|>|x|$. y was taken as a measure of hydration, and as y varies, so will $x(y)$ according to model rules. In the sphere-box model, in focus here, the GAGs incorporate effects from both spherical- and box shape. Electric negative fixed charges of GAGs are distributed either evenly over the GAGs or only over the GAG surfaces or extremities. Cations and ions constitute clouds within ECM cells, but *outside* the regions occupied by GAGs. The ECM cells also hold moving neutralizing charges in regions outside the GAGs. Thus, ECM cells are overall electrically charge neutral, and interact with one another via the collagen network. In between the ECM cells, neutral and electrically charged macromolecules can find their way along different pathways through the tissue: Charged particles only in between GAGs, neutral ones can also diffuse through the GAG regions.

The fixed charges of the GAGs create a self-expansive electrostatic force within each ECM cell, which is transferred to the collagen part of the ECM cell via a hydration dependent GAG-collagen interaction force such that the GAG part and the collagen part will expand differently depending on the degree of hydration. These internal relative movements of ECM cell parts taking place between equilibrium states were a key factor in modeling charged macromolecular exclusion effect at varying degrees of hydration (10). Effects of microscopic GAG expansion and the transfer of force to the collagen part will appear on macroscopic level as a pressure of electrostatic origin on the extracellular matrix. We note that the expansive pressure on collagen part within ECM cells is somewhat reduced because of mobile cations and ions outside the GAG region, and the difference of the dielectric constant value from within to regions outside GAGs also has an effect, in addition to neutralizing charges in the outermost region of each ECM cell. In sum, pressure of electrical origin, varying with hydration, and the effect of interstitial hydrostatic pressure on the collagen matrix, is a central part in our development of an equation of state (an interstitial fluid volume – hydrostatic pressure relationship) for skin and muscle. In addition to elastic forces of the collagen matrix, various tensile forces both from inside and outside the tissues may be included. In static and steady states, which we consider, all forces balance against each other, including forces/pressures from the outside on the tissue boundaries. For the derivation of the equation of state, the tissues are considered homogeneous on macroscopic scale, only at boundaries variation are taken into account. Necessary parameters are hydration dependent, but otherwise considered constant. However, since we start with equations including non-uniformities the results presented may be generalized to non-uniform cases, e.g. in dynamic situations when there is fluid flow into and within the interstitium. Here we thus provide a new model with relevance to in vivo experiments of interstitial fluid volume - hydrostatic pressure relationship where both skin- and muscle tissues are combined, in cases of no fluid flow but with the potential to be generalized to include such effects. The modeling is one-dimensional of combined skeletal muscle and skin tissues, but incorporates spherical effects. It may be generalized to more complex tissue geometries. However, the result characteristics of the present model we expect will be carried over to extended models when the same forces are taken account of.

Table 1 in Wiig and Swartz (13) gives us a set of relevant parameters for interstitial tissue fluid volume, collagen-, GAG- and hyaluronan content. However, because our modeling spans over a range of hydrations, these values give only a limited range of parameter values for skin and muscle. Furthermore, for comparing our model results with in vivo experimental data we need a transformation between model hydration, such as half thickness y of ECMs, which is compatible with

variables used in physical laws, and experimental hydration, such as the ratio ”interstitial fluid volume (ml)/wet weight (g)” of tissue, or ”interstitial fluid volume (ml)/dry weight (g)” This we obtain introducing extended ECM cells in the modeling, ECM+ cells. These new ”building blocks” of tissue also incorporate ordinary cell fluid volume, in particular for muscle tissue, and other solid materials in addition to collagen that constitute the ECM. Cells are assumed for tissue to have a fluid volume content that is a constant ratio of the interstitial fluid volume at every hydration. The elements in the model are schematized in Figure 1, part A showing to the right a macroscopic sketch of a volume without cells but packed with ECM cells representing skin, to the left a similar sketch of muscle tissue including muscle cell fibers- and fluids and vascular networks, and space in between holding similarly sized ECM cells as them to the right. The ratio of muscle cell fluid volume (and we may add vascular system fluids) to interstitial fluid volume is f_c, say 3 (Figure 1B), while small at right, or 0 in a limit case, see below, for skin. In Figure 1, part B, an ECM+ cell for muscle then contains 1 ECM cell plus the number f_c of similar sized cell fluid parts, in addition to solid material, while for skin it is close to $(1+f_c)$ ECM cells. This gives a schematic picture of tissue on macroscopic and microscopic levels where collagen network and GAGs are in contact throughout, and fill space, outside cells and vascular networks, but interact with these fluid compartment tissues. In this way, we shall obtain an easy transformation between model hydration and experimental hydration and vice versa for each tissue type.

It should be noted that the parameter values chosen here are representative for normal tissues such as those studied in the laboratory experiments the present modeling is meant to bridge. The ECM+ way of modeling tissue on microscopic values may, however, also be used for solid cancers, but then with varied sets of parameter values. Varying the f_c - value will scale up or down cell fluid content compared to interstitial fluid volume, and parameters we later introduce for collagen content (f_s), and for cell and vascular solid mass may also be chosen to reflect cancerous but not healthy tissues. For malignant tissues, we can also extend the ECM+ cell concept to a ECM++ cell to encompass tissues containing healthy as well as malignant cells.

To summarize; Electrostatic expansive pressure of GAGs in ECM cells is set up for skin and muscle based on previous models(10) on microscopic level. This pressure will generally vary also on macroscopic scale, and act on the collagen part of the ECM together with collagen elastic and other forces. In particular, there is also hydrostatic fluid pressure influence due to changes of collagen density even if the hydrostatic pressure is homogeneous inside tissue and hence no fluid flow. This effect is mediated through boundary effects. In general we have a two scale situation, where effects on microscopic scale influence or ”feed” effects on macroscopic scale. From the macroscopic equation for the ECM-matrix, incorporating boundary conditions, the interstitial fluid volume-hydrostatic pressure relationships emerge for skin and skeletal muscle. The analysis is thereby generally complex, but we will simplify while highlighting the relevant forces necessary for the derivation of the equations of state, and we leave some mathematical transformations to an Appendix.

Experimental and theoretical hydration parameters

Experimental hydration parameters

In our model as well as in vivo, collagen is a fundamental component of the ECM, being intertwined by GAGs and interacting with them via fixed electrical charges on GAGs through a model collagen-GAG hydration-dependent force. Thereby collagen opens up or closes in a characteristic way in accordance with degree of hydration, closely related to interstitial fluid content. Muscle cell fibers tied to the ECM during variation in hydration, and their fluid contents, play a passive role in these movements, but do transfer pressure between ECM regions. However, experimental hydration markers, or parameters, that have been used, include effects both of fluid contents and solid mass in different ways. These parameters are not directly applicable in mathematical modeling based on physical laws. To omit this problem, we shall use *y* instead, the half thickness of ECM cells we define. Doing so, it becomes important to set up links between this parameter and the experimental ones to be able to compare model with laboratory results.

For this we consider a tissue volume V (either skin or muscle), a sum of an interstitial fluid volume part V_{if} , a cell fluid volume part V_{cf} , a vascular fluid part V_{vf} and a dry, solid volume part V_{dry} , $V = V_{if} + V_{cf} + V_{vf} + V_{dry}$. Here the dry part is considered a sum of a matrix part (collagen part) V_{col} , a vascular dry part V_{vdry} and a cell dry part V_{cdry} , $V_{dry} = V_{col} + V_{vdry} + V_{cdry}$. Hydration parameters of tissues in laboratory experiments we consider have been defined as ”interstitial fluid volume (ml))/(dry weight (g)” e.g. (5, 14), which we term as $W1$, and as the ratio ”interstitial fluid volume (ml))/(wet weight (g)” (3) which we term as $W2$. These are the main parameters besides interstitial fluid hydrostatic pressure being measured in vivo experiments we refer to, giving the measured interstitial fluid pressure-hydration-results. For simplicity, we shall assume specific weights of all fluids to be ρ_f and of all solids ρ_{dry} . Then the mass of volume V is $M = \rho_f (V_{if} + V_{cf} + V_{vf}) + \rho_{dry} V_{dry}$, and the hydration parameters,

$$W1 = V_{if} / (\rho_{dry} V_{dry}) \quad (1)$$

and

$$W2 = V_{if} / M . \quad (2)$$

Then $W2 = (\rho_{dry} V_{dry} / M) W1$, and assuming vascular fluid content is low compared to cell fluid content such that

$$V_{cf} + V_{vf} \approx f_c V_{if} \quad (3)$$

where f_c is a constant factor, say 3 for muscular tissue, close to 0 for skin, then $W2$ can further be expressed by $W1$ as

$$W2 = W1 / (1 + (1 + f_c) \rho_f W1) . \quad (4)$$

ECM and ECM+ cells, and model microscopic hydration parameters

We now make a connection between the model microscopic hydration y and the macroscopic experimental hydration $W1$, and $W2$ in Eqs.(1) and (2).
For this we look more closely into two microscopic model ’cells’, ECM and ECM+, besides ordinary cells, Figure 1:

First of all, ECM cells for the interstitial microscopic volumes, where collagen, GAGs and interstitial fluid interact, give the characteristic expansions of the medium that imparts the whole tissue, ordinary cells included, at varying degrees of hydrations. When ECM cells vary in size due to hydration variations only in "thickness" from y to – y, symmetrically around the mid-point, while having constant cross sectional areas $A = z^2$ perpendicular to y, because of constraints in these transverse directions, then y will be used as a measure of hydration in modeling because it more naturally can be incorporated into physical force laws. These laws determine the extension and internal movements of different parts of ECM cells and also movements on macroscopic scales, and take in particular account of the combined effect on the collagen matrix of fluid hydrostatic pressure, elastic forces, electrostatic forces due to fixed charges via the GAG-collagen interaction forces, and also force due to moving cations and ions within the interstitium. Half ECM cell thickness, or 'hydration' y, varies from a lowest value y_0 to a maximum value y^*, $y^* > y_0$. (y^* and y_0 may both be chosen arbitrarily. In computer runs later we set $y_0 = 1$ and $y^* = 4$, compared to values 1 and 3 in (10). A fractional part of y_0, $f_s y_0$, is its solid part, collagen, per unit cross-sectional area (per U.A.), $v_{col} = f_s y_0$. The second type of 'cell' we construct in each tissue, skin or muscle, the ECM+ cell, is an extension of the ECM cell, and concerning fluid- and material distributions, a miniature copy of the macroscopic tissue: It includes cell- and vascular fluids and their solid materials in addition to interstitial fluid, collagen and GAGs of the ECM cell. ECM+ cells make a coupling between microscopic level and macroscopic / experimental laboratory level easier: Between the macroscopic interstitial fluid volume V_{if} and the corresponding interstitial fluid volume of half an ECM cell we have a macroscopic to microscopic correspondence, ~, $V_{if} \sim y - f_s y_0$ per U.A. Then $W1 \sim w1$,

$$w1 = (y - f_s y_0)/((f_s y_0 + v_{cvdry})\rho_{dry}) \qquad (5)$$

where per U.A. $v_{cvdry} \sim V_{vdry} + V_{cdry}$, the sum of cell and vascular dry material of half an ECM+ cell. Using the correspondence $v_{cf} + v_{vf} \approx f_c v_i = f_c (y - f_s y_0)$ per U.A., the ECM+ version of Eq.(3), corresponding to formula Eq.(4) we have

$$\begin{aligned} w2 &\sim w1/(1+(1+f_c)\rho_f w1) \\ &\sim (y - f_s y_0)/((f_s y_0 + v_{cvdry})\rho_{dry} + (1+f_c)(y - f_s y_0)\rho_f) \end{aligned} \qquad (6)$$

The formulas Eqs(5) and (6) give the relationships between $w1$ and $w2$, representing and corresponding to the macroscopic hydrations $W1$ and $W2$, and the microscopic hydration y.

Incorporating experimental data into hydration parameters

Table 1 in Wiig and Swartz(13) gives some parameter-data for skin and skeletal muscle with relevance to our model, primarily on the macroscopic level. Our model generally spans over a wide range of hydrations for skin- and muscle, both on the microscopic level, i.e. ECM and ECM+ levels, and on the macroscopic level. The table gives, however, the order of magnitude of parameter valuable for setting realistic model parameters values also on the microscopic level. In particular, we note that interstitial volumes V_{if} for skin and muscle in the table are 0.4ml/g ww and

0.1ml/g ww. These values reflect the large amount of cell fluid volume in muscle compared to skin tissue. In the basic modeling presented, we may use identical ECM cells for skin and muscle tissue, and set the f_c-factor equal to 0 for skin and 3 for muscle tissue.

The second experimental observation we shall incorporate is given in Wiig and Reed (5) and Reed and Wiig (14): The values of the hydration parameter used, the ratio "interstitial fluid volume (ml)/ dry weight (g)", for skin and muscle is twice larger for skin: In the modeling where ECM+ cells are central, 1 half ECM+ cell for skin holds 4 similar half ECM cells but negligible cell fluid and solid material apart from collagen, such that $w1 \approx (4\cdot(y-f_s y_0)/(4\cdot\rho_{dry} f_s y_0))$ for skin. A similarly sized half muscle ECM+ cell, however, holds 1 half ECM cell and 3 cell/vascular parts containing in all approximately an interstitial fluid volume per U.A. $(y-f_s y_0)$, and of solid volume, collagen, $f_s y_0$, plus 3 similarly sized solid cell/vascular part, in all $(f_s y_0 + 3\cdot v_{cvdry})\rho_{dry}$ per half ECM+ cell of solid mass per U.A. Therefore, having $3\cdot v_{cvdry} = f_s y_0$ gives a $w1$ for skin tissue twice the corresponding value for muscle tissue, in close accordance with observations(5, 14).

Figure 2 shows relations between hydrations *y*, *w1* and *w2* for skin and skeletal muscle as y varies from $y_0 = 1$ to $y^* = 4$. Besides value of f_c given above, f_s was given the value 0.75. In the rest of the presentation densities of fluids and dry masses all will be set to 1g/ml.

Other parameters:
Fluid pressure and collagen volume fraction

Fluid hydrostatic pressure in the interstitium may be measured relative to the atmospheric pressure p_A, and set to zero if it does not deviate from that, $p_{if} = p_A + p_{if}^{'}$, $p_{if}^{'}$ may be a few mmHg below or above zero. The fluid force per unit volume on solid material in ECM cells (i.e. collagen) of a box-shaped tissue sample varying in the ξ- direction, is $-\partial(ap_{if})/\partial\xi$ where the parameter *a*, a collagen volume fraction, *a* = (collagen volume)/(collagen+fluid volumes).

This parameter value can be determined from the microscopic parameters above for ECM and ECM+ cells in our modeling,

$$a = f_s y_0 / y \qquad (7)$$

being it for skin or muscle tissue ECM+ cells (which holds respectively 4 and 1 ECM cells) On ECM cell scale *a* is constant for constant hydration, y, but decays with increasing hydration, and may in addition vary if f_s varies on macroscopic scale.

Electrostatic pressure on collagen matrix for sphere-box model
GAG radius variation with hydration

An expression of GAG radius variation with variation of hydration is found through a steady state force balance between a self-expansive electrostatic force of GAGs and a GAG-collagen interaction force.

Within a unit surface element of a GAG of radius $R(y)$ in the sphere box model, we have the summed up, absolute value of the electrostatic self-expansive force on the GAG,

$$F_Q = F_{Q1} - F_{Q2} = (3/(64\pi^2\varepsilon_0\kappa_1) - (\kappa_1/\kappa_2)/(32\pi\varepsilon_0\kappa_1))(Q^2/R^4) \quad (8)$$

see Øien and Wiig (10). Q is the total GAG electric fixed charge, which in the model presented is distributed evenly over the spherical GAG region. κ_1 and κ_2 are dielectric constants on the inside (1) and outside (2) of the GAG, and $\kappa_1 < \kappa_2$ since water molecules are more orderly oriented on the outside than on the inside. The first term stems from the self-electrostatic expansive-pressure from inside the GAG, the second from the counteractive pressure from anions and cations outside the GAG-region, where also neutralizing charges move, so that each ECM in all is electrically neutral.

The GAGs in turn interact with the collagen matrix as GAGs are located on the matrix and will expand as hydration changes: The GAG-collagen interaction force within the GAG we modelled spherical symmetric and evenly distributed over the GAG with a force density $k_{f,Sph}(y)$,

$$k_{f,Sph}(y) = k_{f,Sph}(y^*)/(1-(1-b)(y-y^*)^n/(y_0-y^*)^n)^5 \ , y_0 \le y \le y^* \ , n = 1,2,... \quad (9)$$

a function of hydration y, where $b = (k_{f,Sph}(y^*)/k_{f,Sph}(y_0))^{1/5} < 1$, and the GAG-collagen interaction follows an actio-reactio principle : The force on the GAG and the force on collagen are in opposite directions where they act. $k_{f,Sph}(y)$ is a decreasing function of y as y increases from y_0, the lowest hydration, to y^* , the highest hydration, with maximum and minimum values $k_{f,Sph}(y_0)$ and $k_{f,Sph}(y^*)$, Figure 3B. Varying the parameter n gives various shapes of the GAG-collagen interaction force density curve $k_{f,Sph}(y)$ between maximum and minimum values that can reflect differences in tissues being modeled. High values of n means sharper decrease at low hydrations than low values of n.

For the summed up, absolute value of GAG-collagen interaction force within a unit surface element of the spherical GAG we then have,

$$F_{\text{int}} = (R/3)k_{f,Sph}(y) \quad (10)$$

Hence a GAG force balance on the average can be expressed as,

$$F_Q - F_{\text{int}} = 0 \quad (11)$$

giving

$$R^5(y) = k_Q / k_{f,Sph}(y) \quad (12)$$

where $k_Q = (9-6(\kappa_1/\kappa_2))Q^2/(64\pi^2\varepsilon_0\kappa_1)$ has physical dimension 'force times length squared', and hence for the GAG radius at hydration y,

$$R(y) = KQkf(1-(1-b)(y-y^*)^n/(y_0-y^*)^n) \ , \ y_0 \le y \le y^*, n = 1,2,... \quad (13)$$

Here $KQkf = (k_Q/k_{f,Sph}(y^*))^{1/5}$. Note that $KQkf$ varies with charge as $Q^{2/5}$ and with $k_{f,Sph}(y^*)$ as $k_{f,Sph}^{-1/5}(y^*)$.

Simplifying characteristics are:

$KQkf = R(y^*)$ and $k_{f,Sph}(y_0)/k_{f,Sph}(y^*) = (R(y^*)/R(y_0))^5$, (14)

and hence

$b = R(y_0)/R(y^*)$. (15)

For n=2 and n=8 Figure 3A demonstrates we have sharper rise for $R(y)$ when $k_{f,Sph}(y)$ shown through $(k_{f,Sph}(y)/k_{f,Sph}(y^*))^{1/25}$ in Figure 3B has sharper fall, in accordance with Eq.(12). We note that in the sphere box model, GAG expansion given by $R(y)$ is modified by a factor approximately ½, as we shall see below, due to constraints in directions transverse to the thickness direction.

Electrostatic pressure on collagen in the sphere-box model

In the model presented, the spherical GAGs on microscopic scale manage to expand the collagen matrix, via the GAG-collagen interaction force, mainly in the y-directions (positive and negative) because there are assumed constraints and negligible movements of collagen transverse to these directions. Effects of a weak bending and tension of collagen fibers in transverse y directions will be taken account of later. Hence we get a sphere box model with a GAG box of half box thickness $\alpha R(y)$, where $\alpha=\pi/6$, and cross sectional area z^2, see Figure 1, part C, and with smeared out interaction force density in the y-directions, positive and negative, on the collagen part within a flat GAG-region, see the Appendix and (10), given by

$$\overline{f_{y,\mathrm{int}}} = (9-6(\kappa_1/\kappa_2))Q^2/(32\pi^2\varepsilon_0\kappa_1 R^3(y)z^2) \quad (16)$$

The force spreads via collagen from ECM cell to ECM cell to all parts of the matrix, and manifests itself as a volume force on macroscopic scale acting on the elastic collagen matrix that can be derived from an electrostatic pressure. The electrostatic pressure is defined as an average of the force acting over transverse to y surfaces within the ECM cell, and is derived in the Appendix. We get for this pressure for both skin and muscle

$$\tilde{p}_E = \frac{(\alpha R(y))^2}{2y}\overline{f_{y,\mathrm{int}}} = \mathrm{K}((\pi/6)^2/2)Q^2/(yR(y)z^2) \quad (17)$$

where $K = (9-6(\kappa_1/\kappa_2))/(6\cdot 32\varepsilon_0\kappa_1)$ may vary with dielectric constants. (Note difference here and in the Appendix between the averages $\bar{h}$ (over right half GAG box) and $\tilde{h}$ (over ECM). The pressure takes account of both spherical- and box effects of the ECM cells: Q^2/z^2 is the box effect, see (10) , $1/R(y)$ the spherical effect, and the pressure varies with Q as $Q^{8/5}$.

In this basic model, the electrostatic pressures for skin and muscle tissue have the same values: Their values originate on microscopic scale of similar ECM cells of same hydration y having the same fixed electric charge and collagen content. Then GAG radii of skin and muscle ECM cells span over the same range as y-value varies in the model. There are 4 times as many skin ECM cells as there are muscle ECM cells in equal sized volumes, but densities of ECM cells in skin and muscle interstitial volumes are practically the same in the model. While in a limit case skin has no ordinary cells to be filled with cell fluid, each ECM of muscle ECM+ cell on the average feeds a 3-fold of similarly sized ordinary cell with cell fluid volumes. While in the model skin ECM cells interact between each other ”undisturbed” by ordinary

cells, muscle ECM cells interact partly directly, partly via ordinary cell regions in between, with no loss of effect.

When comparing with experimental hydrostatic interstitial fluid pressure-hydration relationship data, instead of *y*-dependence in each tissue, we transform in Eq.(17) to hydrations $w1$ and $w2$ via the inverses of Eqs.(5)-(6), corresponding to $W1$ and $W2$.

RESULTS AND DISCUSSION

Equation of state of the interstitium

The preceding sections give the basis for setting up the equations of state for skin and muscle interstitia. The framework for this shall be the steady state, one dimensional collagen equation

$$\partial(\kappa_e \partial u/\partial\xi)/\partial\xi - \partial p_E/\partial\xi - \partial(ap_{if})/\partial\xi + K_D v_{if} = 0 \qquad (18)$$

where terms are: The elastic force due to compression/extension in y-directions, the electrostatic- and hydrostatic forces, and the drag force due to streaming of the interstitial fluid with velocity v_{if}. An extra tensile force due to weak tension and bending of collagen fibers directed transverse to *y* that influences *y*-directed extension could be added on the left side of Eq.(18), but will instead be lumped together with a similar force due to the outermost skin extension that comes into play later. κ_e is the elastic modulus, *a* is given from Eq.(7) and K_D is the Darcy constant: The drag force term is coupled to the Darcy law

$$-\frac{\partial p_{if}}{\partial\xi} - K_D v_{if} = 0 \qquad . \qquad (19)$$

The equations to some extent generalize equations used elsewhere, mostly in cartilage tissues, e.g.(15), with hydrostatic pressure loading as in(16). This is because terms of the collagen equation Eq.(18) vary on both microscopic (ECM/ECM+) and on macroscopic scales, i.e. the sizes of skin- and muscle tissues, and includes the particular electrostatic force term. Space variables on the two scales will be denoted ξ_0, on microscopic scale, and ξ_1 on macroscopic scale. These variables are related by $\xi_1 \approx \varepsilon\ \xi_0$, where the parameter $\varepsilon << 1$ is the ratio between microscopic and macroscopic scales. For small variations of ε, ξ_0 and ξ_1 may be considered independent variables(17). Then $\partial/\partial\xi \approx \partial/\partial\xi_0 + \varepsilon\partial/\partial\xi_1$ in Eq.(18), while $\partial/\partial\xi \approx \varepsilon\partial/\partial\xi_1$ in Eq.(19) because of smallness of velocity v_{if} that will be assumed. In addition to the drag force term also the hydrostatic pressure term in Eq.(18) will be assumed small, of order ε, compared to the elastic term and the electrostatic force term which vary on both scales. In the Appendix equations to zeroth and first order in the smallness parameter ε are set up. An average of the equation to first order over the microscopic scale is performed in order to incorporate effects from the microscopic scale in tissue into macroscopic scale. Taking then account of effects stemming from the outermost regions of tissue, its boundaries, we thus couple effects on both microscopic and macroscopic scales to effects from tissue surroundings. When also fluid flow is nulled out, which conforms with in vivo physiologic experiments (5, 14), we are left with two main cases of effects on tissue state, see

Figure 7: The first is connected to the case when collagen ends on microscopic ECM scale in the lowest order (zeroth order in ε) equation are considered "free", meaning $\partial u/\partial \xi_0 = 0$ at $\xi_0 = \pm y$, for every hydration *y*, the second case when ends are fixed , $u = 0$ at $\xi_0 = \pm y$ instead. Then inside tissue on macroscopic scale, when $v_{if} = 0$, we have from the first order Eqs,(A8)-(A9), on macroscopic scale

$$ap_{if} = c_1, \tag{20}$$

in the first case, and

$$\tilde{p}_E + ap_{if} = c_2, \tag{21}$$

in the second case. The c's are constants in both cases at every hydration *y*, $y_0 < y < y^*$. The effect of the electrostatic pressure only shows up in the second case, because when ends are free in the first case, electrostatic force and elastic force on the average over each ECM cell cancel out, see the Appendix.

At an outermost skin tissue boundary, these pressures effectuated by the collagen network must balance pressures from outside on the network through the interface: With p_A the atmospheric pressure and S a pressure due to skin tension, pointing inwards, pressure on collagen from outside is $ap_A + S$, and we have $p_{if} = p_A + S/a$ from Eq.(20) and $p_{if} = p_A - \tilde{p}_E/a + S/a$ from Eq.(21). As noted above we now incorporate into *S* the weak tensile force due to bending of collagen fibers that also are directed inwards. Measuring then fluid hydrostatic pressure deviations from p_A, $p_{if} = p_A + p'_{if}$, then

$$p'_{if} = S/a \tag{22}$$

in the first case, and

$$p'_{if} = -\tilde{p}_E/a + S/a \tag{23}$$

in the second case, where $\tilde{p}_E$ is given from Eq.(17) and *a* from Eq.(7). Inserting from Eq.(17) in the second case,

$$p'_{if} = \left(-\mathrm{K}((\pi/6)^2/2)Q^2/(R(y)z^2) + S\cdot y\right)/f_s y_0. \tag{24}$$

The hydrostatic pressure continues into the muscle tissue, if no extra further inner tensions are set up there, since skin and muscle ECM's in the basic model are identical, with same *y*-value, same *a*-value, same elastic modulus of collagen and same fixed charge value. Difference in tissues lies in different ECM+ cells (Figure 1B), and therefore in hydration when hydration is measured in "ml per gram wet weight" or "ml per gram dry weight". Thus, from the skin/muscle collagen interphase with no extra tensile forces in addition to *S* there, the balance in hydrostatic pressure and electrostatic mean pressure may spread into the central muscle region.
The lumped tensile force S will be assumed a function of hydration *y*: From the lowest hydration y_0 (a dehydrated state) we assume the tensile force first decays as

$$S = s_0/y \tag{25}$$

as y increases up to $y = y_L$ below y*. Then $S\cdot y = s_0$, in Eq.(24). From there on S may change smoothly into a branch that tend to increase instead, e.g. like $\approx 1/(y^* - y)$ as hydration further increases towards *y**. This increase may be similar to observed skin behavior at edematous states (18).

In the ”free end case” electrostatic pressure balances elastic contraction force in thickness direction, see the Appendix. Hydrostatic pressure will then take the value Eq.(22), $S/a = s_0/(f_s y_0)$.

In the ”fixed end case” electrostatic pressure stemming from GAG electric charge adds up to hydrostatic pressure and in sum counteract pressures from the lumped tensile forces, Eq.(23)/Eq.(24). This case seems to be the model case that can explain physiological experiments given in (5, 14) and (3), and model results are given in Figures 4-6, and can be compared to the experimental results:
Figures 4 and 5, show hydrostatic pressure from Eqs.(23)/(24) as hydration *w1* (*w1* as function of *y* is given from Eq.(5)) increases, both without and with tensile force S. The lumped tension term in the model is given the value $s_0/(f_s y_0) = 3.75$ for skin and muscle tissues, while the parameter $\mathrm{K}((\pi/6)^2/2)(Q^2/z^2)/(f_s y_0)$ has been given an appropriate value, at first 10. Experimental curves from Wiig and Reed (5) for skin and from Reed and Wiig (14) for skeletal muscle are shown in Figures 4B and 5B, respectively, for comparison with model predictions. Later experiments by Wiig and Reed (19) for skin and skeletal tissues with alternative techniques have verified these results. The match between model and experimental curves for skeletal muscle Figure 5B is quite good for response factor n=8. For skin, Figure 4B, the match is overall good in this basic and first modeling. Generalizing the model to have different ECM cells for skin and skeletal muscle tissues may improve the match even more. Effect on interstitial hydrostatic pressure of varying GAG charge Q is also demonstrated: Electrostatic pressure in the model varies with charge as $Q^{8/5}$, and curves for a Q-value in accordance with parameter values above and for a Q reduced by a factor 0.8 are shown. Difference in skin and muscle curves is mainly due to muscle solid mass in our model, and in agreement with experimental results(5, 14). Model curves when fixed charge Q changes are also in accordance with in vivo data(3). Please also observe that the model predicts that lowering of $f_s y_0$ only in Eq.(24), and s_0 in Eq.(25) is constant, will lift the positive part of hydrostatic pressure curve and lower the negative part and therefore has similar effect as a change in GAG charge.
In Figure 6B, effects of increasing GAG-collagen interaction force density is shown and compared to previous values and curves; While $k_{f,Sph}(y_0)$ was the same, $k_{f,Sph}(y^*)$ was increased by a factor 5 of value used in previous figures. Figure 6A shows why; the increase of $k_{f,Sph}(y^*)$ gives an overall increase of $k_{f,Sph}(y)$ that counteracts GAG self-expansiveness, hence we get a smaller GAG radius $R(y)$, meaning a larger $1/R(y)$ and then larger $\tilde{p}_E$ from Eq.(17), and $-\tilde{p}_E$ will fall below the smaller $k_{f,Sph}(y)$-case.

SUMMARY AND CONCLUSIONS

Here we have presented a new physical/mathematical model for the relationship between interstitial fluid volume and hydrostatic pressure in skin and muscle interstitium reflecting macroscopic (millimeter –to centimeter scale) in vivo experiments on rats (3, 5, 14). The model extends a previous model of tissue hydration effects on volume exclusion of electric charged and neutral macromolecules

in skin (10, 20). The presented model includes parameters that are determinants of the shapes of curves in pressure-volume diagrams, and is based on effects from both microscopic scale (nano- to micrometer) and macroscopic scale. Model results comply very well with these experimental data, both for model skin and skeletal muscle. Moreover, we are able to demonstrate how microscopic mechanisms are "reflected into" macroscopic effects. The in vivo experiments were performed on a large number of similar individuals at varying degrees of hydration, and the experimental hydration-pressure points fall within the areas in the hydration-pressure diagram encompassed by model hydration-pressure curves when model parameters are varied on the microscopic level, for instance amount of GAG electric charge or collagen content of tissues.

As for the in vivo experiments used for comparison, in the models presented there is no net fluid flow. An extension of the model to include fluid transport in the interstitium of skin and muscle along similar lines of previous work (21-24), where the transport between blood and lymph vessels is addressed, as well as transport of therapeutic agents to cells under various degrees of hydration, contents of GAGs, and collagen conditions, will be a natural follow up of our studies.

A novel aspect of the model is to include a GAG-collagen hydration dependent interaction force on microscopic level. This force transfers self-expansive electrostatic forces and electrostatic pressure of GAGs to the extracellular collagen matrix on microscopic scale. In turn, these forces, via an averaging process, generate the macroscopic electrostatic pressure that have to balance in particular hydrostatic pressure and boundary effects on that scale. Apart from amount of GAG charge Q and solid mass, mainly given by f_s, tensile forces from both inside and outside tissue, S, the amount of cell mass and fluids, f_c and strength of GAG-collagen interaction, given by the force density $k_{f,Sph}(y)$ for different values of the parameter n, are all determinants of the shape and placement of the pressure-volume curve in the diagrams. In principle, variations in some of these variables might be tested against in vivo experiments. For living species, individuals may, depending on the condition, shift from one set of parameters to another, such that individuals function in the area between curves, e.g. between n=2 and n=8 as shown in the model. Varying the amount of GAG charge as well as collagen will shift the curves parallel to the fluid pressure axis in accordance with laboratory experiments. Tensile forces on the tissue from outside will lift the pressure-volume curves and result in a hydrostatic pressure at higher hydrations that is above atmospheric pressure. Density of cell- and vascular dry mass will shift hydration differently when measured on a wet weight or dry weight basis. When using ECM and ECM+ cells in the model, the dry weight is most directly related to the model parameter "half thickness" of ECM cells on microscopic scale, see Eqs.(5) and (6) and Figure 2. We have chosen to base the model on dry weight in Figures 4, 5 and 6. The dry weight basis will in particular reflect the cell solid mass of muscle tissue in addition to ECM cells, with its contents of GAGs, collagen and interstitial fluid. The wet weight basis will also reflect the amount of cell fluid. Because the values of GAG charges and densities of collagen may be difficult to determine precisely, while tensile force S from outside may be controlled, comparing laboratory measurements and model results may help us to determine values of GAG charge and density of collagen in various tissues.

As discussed above, the ECM+ cell concept we have developed to bridge experimental and model results of interstitial fluid volume-fluid hydrostatic

relationships, may well be used also for solid tumors by varying parameter values for cell fluid content, solid masses of collagen, cells and vascular-systems. The ECM+ concept may then be extended to an ECM++ concept taking into account both normal and malignant cells and tissue elements at the microscopic level. In this way, considering also interstitial fluid convection and hydrostatic pressure variation on macroscopic scale, not only paths of interstitial fluid through complex tissues can be found at varying degrees of hydration, but also available volumes for various charged and neutral macromolecular therapeutic agents in these tissues, and their transports by convection and diffusion. More insight into mechanisms taking place in the interstitial space will help our general understanding of physiology, and in particular of transport mechanisms of charged and neutral macromolecules between vascular systems and cells. The degree of hydration seems to be an important factor for understanding such mechanisms.

Author Contributions

AHØ and HW conceived the study, AØ performed the modeling, AHØ and HW wrote the paper, AHØ and HW both approved the final version of the manuscript.

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APPENDIX

GAG-collagen interaction force density for sphere box

In Øien and Wiig (10), the following GAG-collagen interaction force density within the GAG box in y-direction for the sphere box model was derived,

$$\overline{f_{y,\mathrm{int}}} = 2k_{f,Sph}(y)R^2(y)/z^2 \ . \qquad \text{(A1)}$$

Using $k_{f,Sph}(y) = k_Q / R^5(y)$ from Eq.(12) it may also be written,

$$\overline{f_{y,\mathrm{int}}} = 2k_Q/(R^3(y)z^2) = (9-6(\kappa_1/\kappa_2))Q^2/(32\pi^2\varepsilon_0\kappa_1 R^3(y)z^2) . \qquad \text{(A2)}$$

By symmetry, the GAG-collagen interaction force density in the left half GAG box is $-\overline{f_{y,\mathrm{int}}}$, pointing leftwards.

The derivation of formula Eq.(A1) went through a series of steps: From 1), the radially directed, smeared-out GAG-collagen interaction force density within the right half of the GAG sphere, $\mathbf{F}_{\mathrm{int}} = k_{f,Sph}(y)\mathbf{r}/r$, to 2), the smeared out force density in the y-direction, $f_{y,\mathrm{int}} = k_{f,Sph}(y)/2$, to 3) replacing the half sphere at any but fixed hydration y with a GAG-box of quadratic bottom of sides 2*R* and thickness αR and with the same expansive force density as previously, such that the total expansive force in the y-direction (and –y-direction) of the GAG-box is the same as for each half sphere. For this α must have the value $\alpha = \pi/6$, to 4) a second GAG-box, see strip boxes of Figure 1, part C, of quadratic bottom sides z instead, $z > 2R$, and the same thickness αR but with a force density $\overline{f_{y,\mathrm{int}}}$ such that the total expansive forces to the right are the same: Thus, in all, this gives $\overline{f_{y,\mathrm{int}}} z^2 \alpha R(y) = 4R^2 \alpha R(y) k_{f,Sph}(y)/2$ and hence Eq.(A1).

Electrostatic pressure

For the expansive pressure due to the GAG-collagen interaction force at the transverse surface at thickness position ξ, $-\alpha R(y) \le \xi \le \alpha R(y)$ we define

$$p_E(\xi) = |\overline{f_{y,\mathrm{int}}} \cdot \xi| . \qquad \text{(A3)}$$

The average of this expansive pressure over the whole of $(-y, y)$ is

$$\tilde{p}_E = (1/(2y)) \int_{-y}^{y} p_E(\xi) d\xi = (1/y) \overline{f_{y,\mathrm{int}}} \int_0^{\alpha R(y)} \xi d\xi = \frac{(\alpha R(y))^2}{2y} \overline{f_{y,\mathrm{int}}} \qquad \text{(A4)}$$

This average expansive pressure over the microscopic ECM cell may vary over a macroscopic scale ξ_1 directed along vector $\mathbf{j}$, say due to variation of fix charge Q, and then the electrostatic force per unit volume on the collagen matrix on macroscopic scale is $-\mathbf{j}\, \partial \tilde{p}_E / \partial \xi_1$.

Averaging Collagen Equation over microscopic scale leading to macroscopic collagen equation

The collagen equation Eq.(18) to zeroth order in ε is

$$\partial(\kappa_e \partial u / \partial \xi_0) / \partial \xi_0 - \partial p_E / \partial \xi_0 = 0 \qquad \text{(A5)}$$

and to first order

$$2\partial(\kappa_e \partial u / \partial \xi_0) / \partial \xi_1 - \partial p_E / \partial \xi_1 - \partial(a p_{if}) / \partial \xi_1 + K_D v_{if} = 0 \qquad \text{(A6)}$$

which is coupled to Eq.(19), where $\partial / \partial \xi \to \partial / \partial \xi_1$. These equations are valid for every hydration y, $y_0 < y < y^*$.

In Eq.(A5), $-\partial p_E / \partial \xi_0 = \overline{f_{y,\mathrm{int}}} H(\alpha R(y))$, (for $\overline{f_{y,\mathrm{int}}}$ see Eq.(A2)) where H takes value 1 for $0 < \xi_0 < (\alpha R(y))$ and zero for $\alpha R(y) < \xi_0 < y$ in the right half ECM cell, and $-\partial p_E / \partial \xi_0$ points opposite for $-y \le \xi_0 < 0$. Eq.(A5), was solved in(10) for the two cases, u fixed and u free at the ends $\pm y$ of the ECM cell, see Figure 7.

Averaging Eq.(A6) over the interval, $-y < \xi_0 < y$ gives the volume density force balance of the collagen matrix on macroscopic scale,

$$\begin{cases} 0 \\ (1/y)\partial(\kappa_e u_m) / \partial \xi_1 \end{cases} \quad -\partial \tilde{p}_E / \partial \xi_1 - \partial(a p_{if}) / \partial \xi_1 + K_D v_{if} = 0 \qquad \text{(A7)}$$

The upper expression in the first term is for the case when on ECM-level collagen ends are fixed, and the lower expression when ends are free, and u_m then is the positive collagen stretch at $\xi_0 = y$ (at $\xi_0 = -y$, stretch is $-u_m$).

$\tilde{p}_E$ in the second term is given by Eq.(17).

From Øien and Wiig (10) we have the formula $u_m = \pi^3 k_Q / (216 \kappa_e \alpha R(y) z^2)$ for every y on ECM-level in the free end case, and hence it turns out that in this case $(1/y)\partial(\kappa_e u_m) / \partial \xi_1 - \partial \tilde{p}_E / \partial \xi_1 = 0$, see also Figure 7, which simply means that in the free end case on microscopic ECM-level electrostatic mean expansive force balances the collagen contractive force. In this case Eq.(A7) reduces to the macroscopic force balance,

$$-\partial(ap_{if})/\partial\xi_1 + K_D v_{if} = 0. \quad \text{(A8)}$$

Of more interest in this paper is the fixed end case. Then the macroscopic collagen equation reduces to the force balance

$$-\partial\tilde{p}_E/\partial\xi_1 - \partial(ap_{if})/\partial\xi_1 + K_D v_{if} = 0 \quad \text{(A9)}$$

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Figure Legends

Figure 1. Schematic figures of distribution of fluids, GAGs, collagen and other solid parts in model skin- and skeletal muscle tissues on macroscopic and microscopic scales, A and B, with symbols given in C: Cross-sectional views of tissues with thicknesses in right and left directions. In C, also indicated ECM cell of thickness 2*y*, in sphere box model with spherical GAG, radius *R*(*y*), and corresponding rectangular GAG in sphere-box model, of half thickness $\alpha R(y)$ ($\alpha = \pi/6$).

Figure 2. Relations between model hydration y (half thickness of ECM-cell on nanometer or micro-meter scale) and hydrations used in laboratory in vivo experiments, w1 (solid lines —) and w2 (dashed lines -----).

Figure 3. A: Variations of GAG radius *R*(*y*) from 0.8 to 3.5 on nanometer or micro-meter scale for two values of response factor *n*, *n*=2 (low response) and *n*=8 (high response) as hydration *y* (half ECM-thickness) varies from $y_0 = 1$ to $y^* = 4$, on the same scale. B: Variation of interaction force density within GAGs, $k_{f,Sph}(y)$, shown through $(k_{f,Sph}(y)/k_{f,Sph}(y^*))^{1/25}$, that balances GAG electrostatic expansive force density. Note rapid GAG expansion at low hydration *y* corresponds to sharper fall of interaction force density.

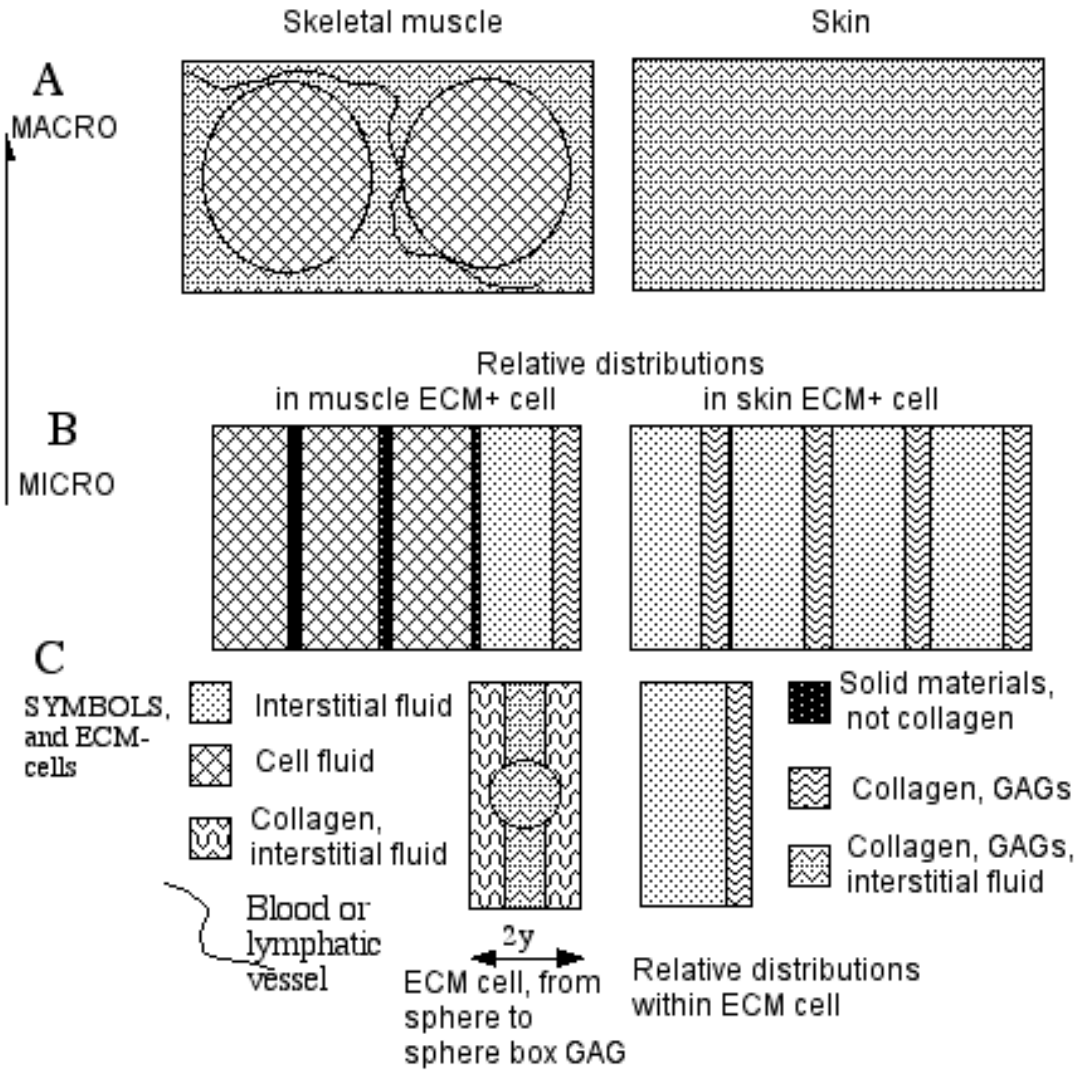
Figure 4. A: Interstitial hydrostatic pressure-fluid volume curves in skin when the tensile force *S* is absent, for two strengths of GAG electric charge. Dashed curves (----) show results when charge has been reduced by a factor 0.8 as compared to charge used for the solid line (—) curves, where the lumped parameter $K((\pi/6)^2/2)(Q^2/z^2)/(f_s y_0)$, see Eq.(24), was given the value 10. Parameter values *n*=2 and *n*=8 refer to slow and sharp GAG radius rise when hydration increases. All values of *n* in this range will fill the space in between the dashed and the solid line curves shown. B: Interstitial hydrostatic pressure-fluid volume- curves in skin when the tensile force *S* has been included (S/a equals $s_0/(f_s y_0)$, and $s_0/(f_s y_0)$=3.75) compared to the in vivo experimental curve in small dots (oooo). Otherwise same parameters used and varied as in A.

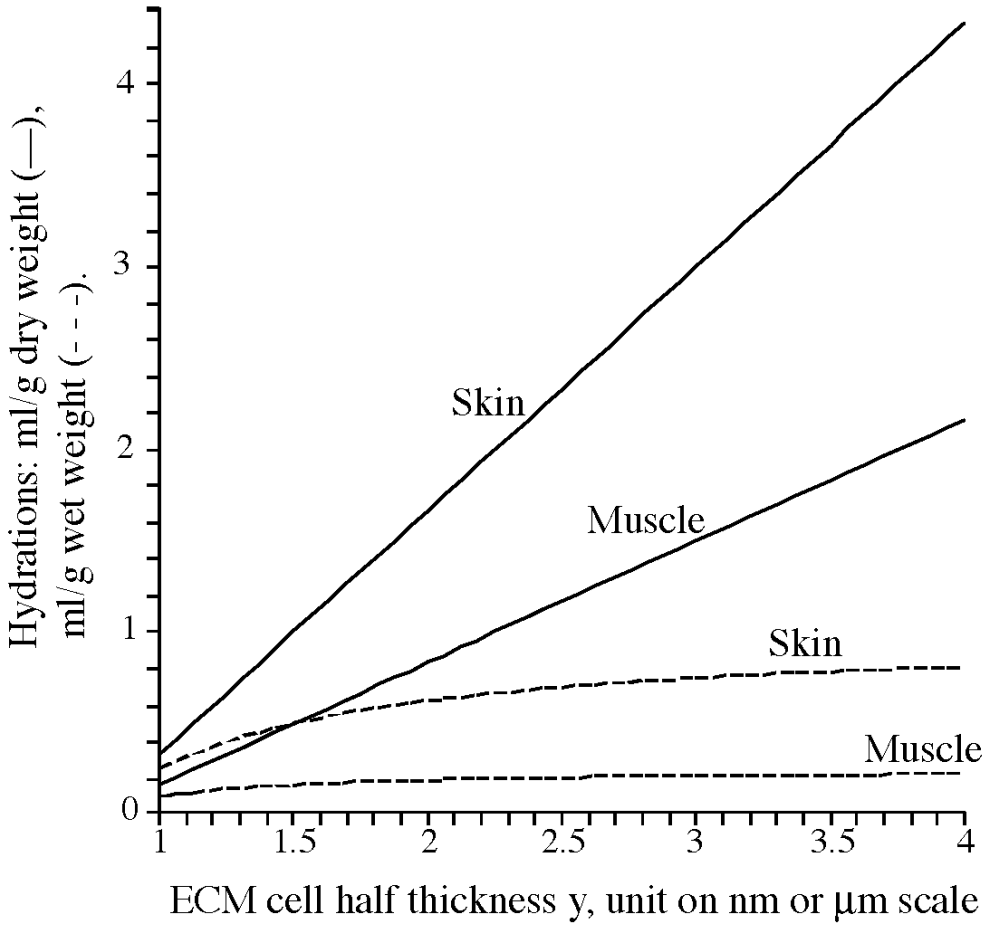
Figure 5. A: Interstitial hydrostatic pressure-fluid volume curves in skeletal muscle when effect of the tensile force *S* is absent, for two strengths of GAG electric charge. Dashed curves (-----) show results when charge has been reduced by a factor 0.8 as compared to charge used for the solid line (—) curves, where the lumped parameter $K((\pi/6)^2/2)(Q^2/z^2)/(f_s y_0)$, see Eq.(24), was given the value 10. Parameter values *n*=2 and *n*=8 refer to slow and sharp GAG radius rise when hydration increases. All values of *n* in this range will fill the space in between the dashed and the solid line curves shown. B: Interstitial hydrostatic pressure-fluid volume- curves in muscle when the tensile force *S* has been included (S/a equals $s_0/(f_s y_0)$, and $s_0/(f_s y_0)$=3.75), compared to the in vivo experimental curve in small dots (oooo). Otherwise same parameters used and varied as in A. Values of hydration changed compared to values of skin, primarily because of effect of cell

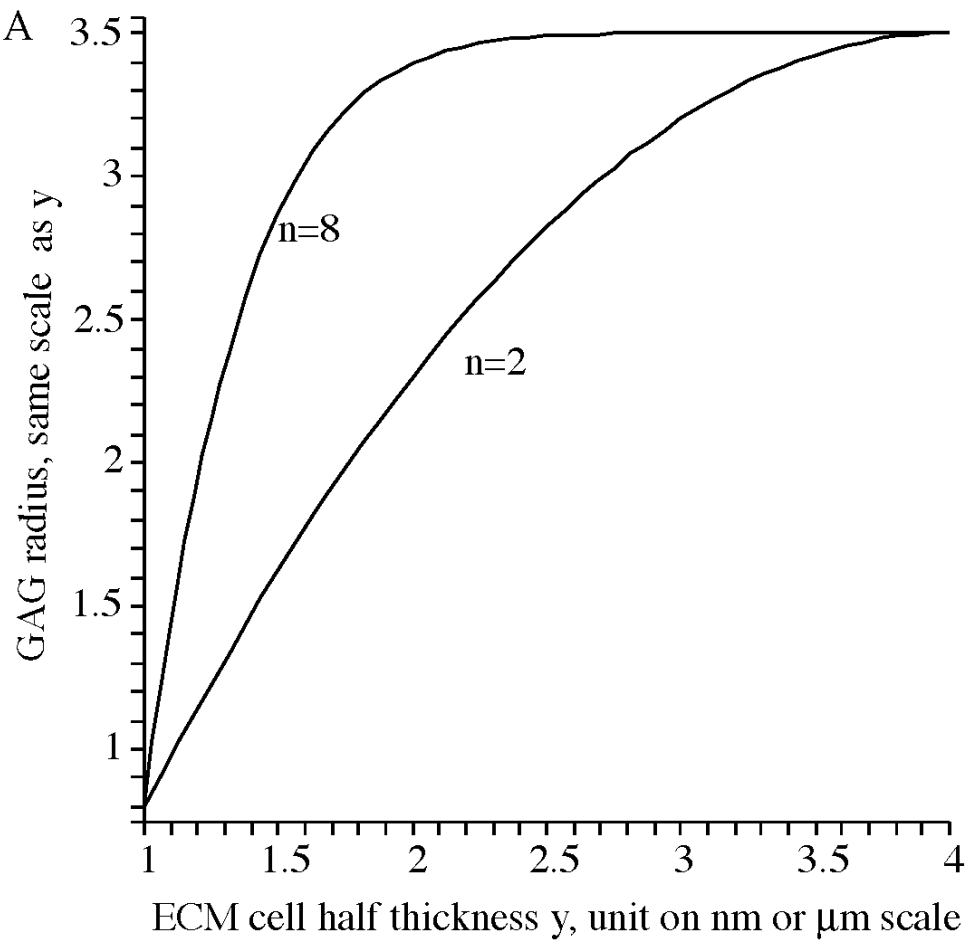
fluid, which was set to zero for skin. Effects of the tensile force *S* is assumed to be transferred unchanged through layers between skin and skeletal muscle tissue.

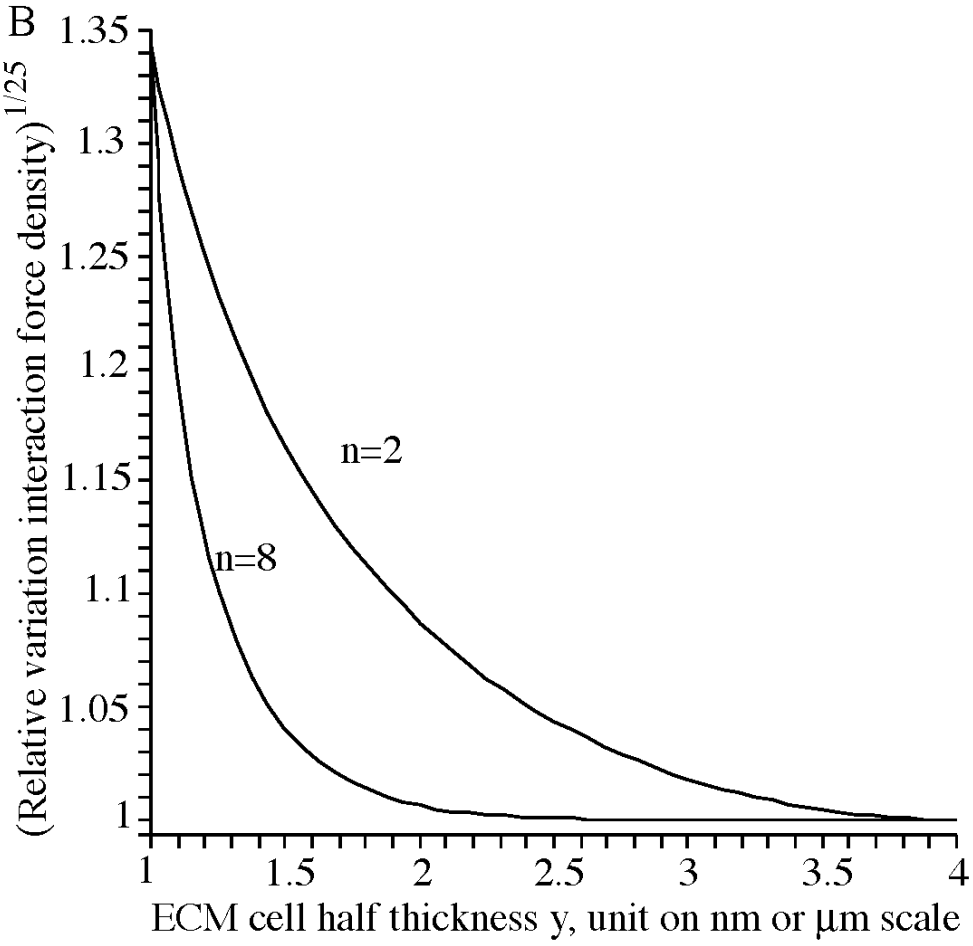
Figure 6. A: GAG radius curve, shown in dots (oooo), when parameter for GAG-collagen interaction density force at highest hydration, $k_{f,Sph}(y^*)$, has been increased by a factor 5 as compared to the value used above, curves Figure 3A, while $k_{f,Sph}(y_0)$ is unchanged. Then $k_{f,Sph}(y)$ has been increased, all y , $y_0 < y \leq y^*$. B: Corresponding interstitial hydrostatic pressure-fluid volume curves in skin when tension used above in Figure 4B is included in dots (oooo). Solid line as in Figure 4B. No reduction of *Q*.

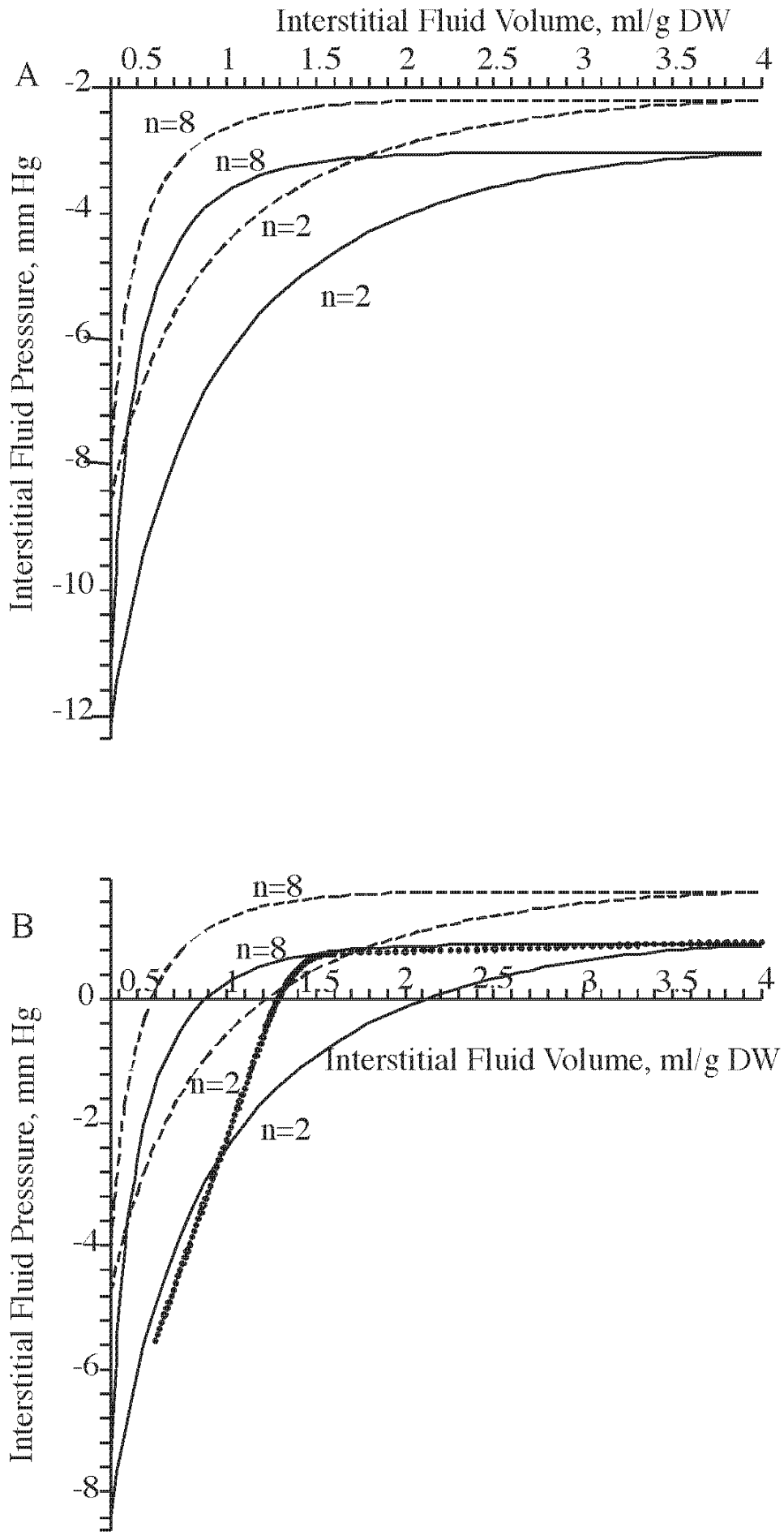
Figure 7: A: Three consecutive ECM cells at a particular hydration (half thickness *y*). B: A schematical display of added up elongations and contractions u on microscopic level of the ECM collagen parts at this hydration. In each ECM cell u results from the intrinsic contractive elastic force of collagen, the expansive interaction force with GAGs, that transfers GAG electrostatic expansion to the collagen of each cell, and interactions between collagen parts of ECM cells (boundary conditions of each cell), with ends fixed or free.

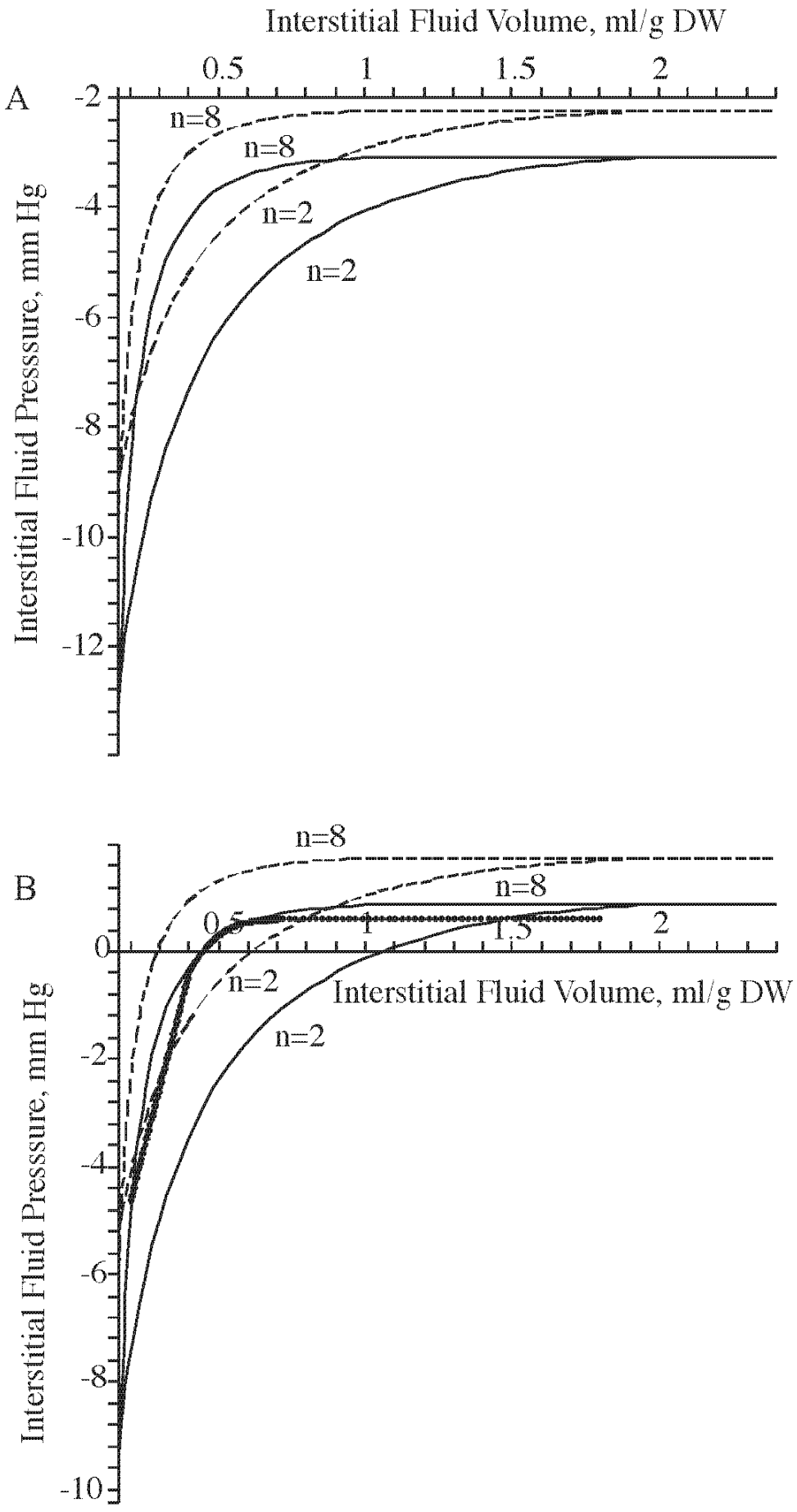

Skeletal muscle
Skin
A
MACRO
Relative distributions
in muscle ECM+ cell
in skin ECM+ cell
B
MICRO
C
SYMBOLS, and ECM-cells
Interstitial fluid
Cell fluid
Collagen, interstitial fluid
Blood or lymphatic vessel
2y
ECM cell, from sphere to sphere box GAG
Relative distributions within ECM cell
Solid materials, not collagen
Collagen, GAGs
Collagen, GAGs, interstitial fluid

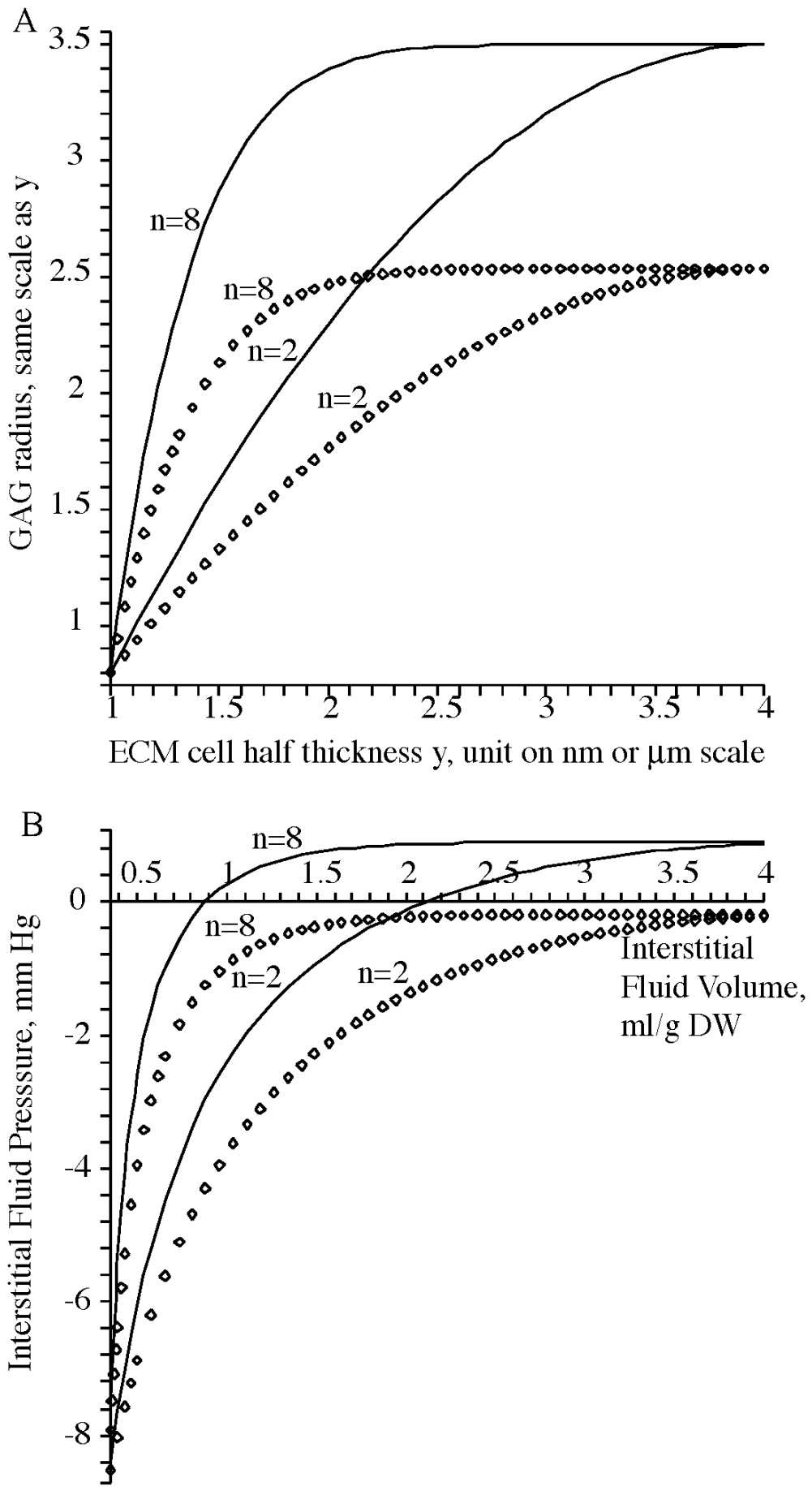

Hydrations: ml/g dry weight (—),
ml/g wet weight (- - -).
0
1
2
3
4
Skin
Muscle
Skin
Muscle
1
1.5
2
2.5
3
3.5
4
ECM cell half thickness y, unit on nm or μm scale

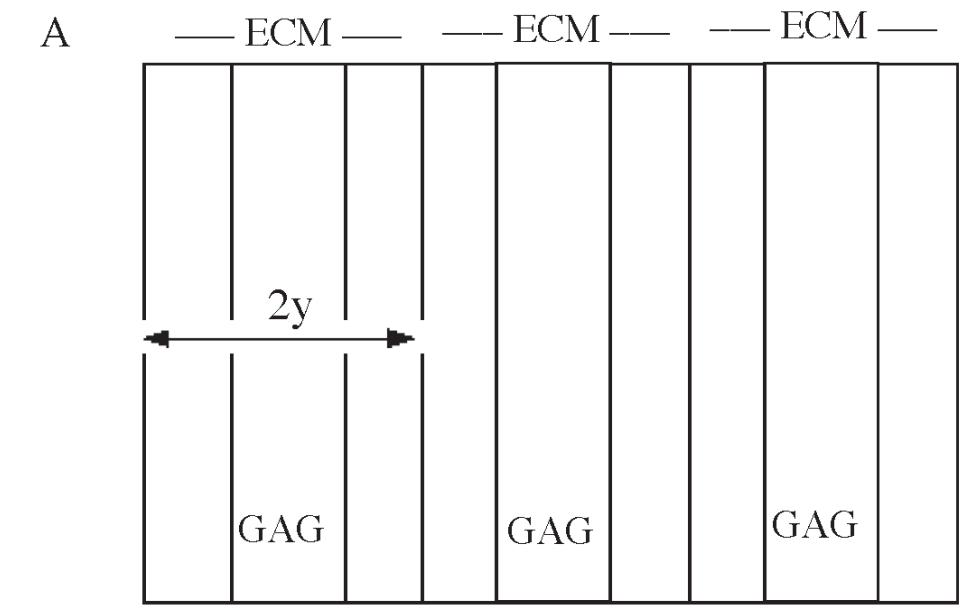

A
3.5
3
2.5
2
1.5
1
GAG radius, same scale as y
n=8
n=2
1
1.5
2
2.5
3
3.5
4
ECM cell half thickness y, unit on nm or μm scale

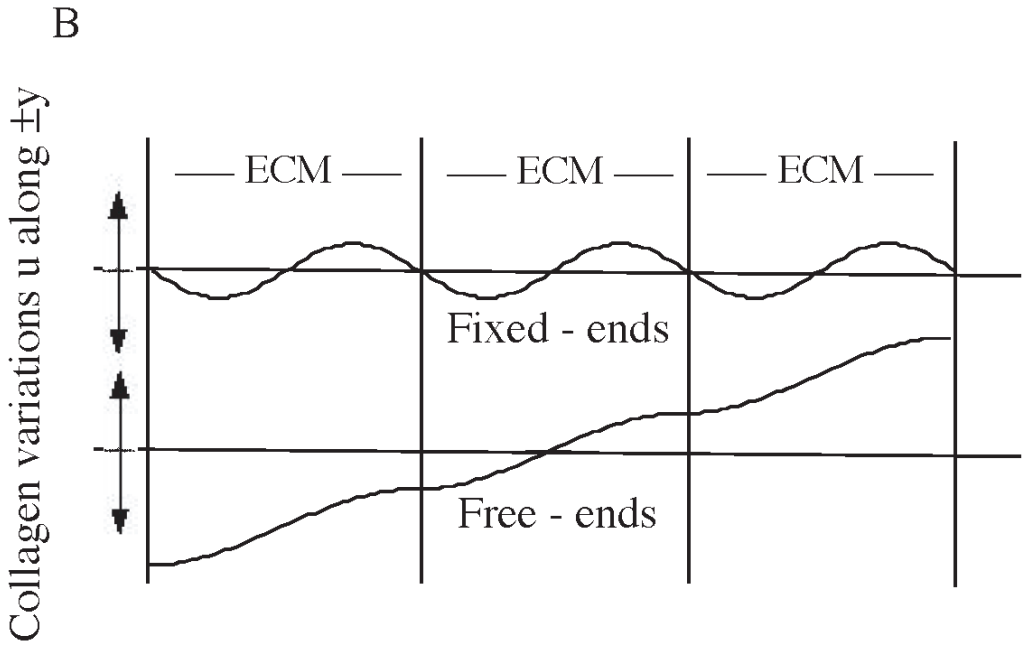

B
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
(Relative variation interaction force density)^1/25
n=2
n=8
1
1.5
2
2.5
3
3.5
4
ECM cell half thickness y, unit on nm or μm scale


A
Interstitial Fluid Volume, ml/g DW
0.5
1
1.5
2
2.5
3
3.5
4
-2
-4
-6
-8
-10
-12
Interstitial Fluid Presssure, mm Hg
n=8
n=8
n=2
n=2
B
n=8
n=8
0
0.5
1
1.5
2
2.5
3
3.5
4
-2
-4
-6
-8
Interstitial Fluid Volume, ml/g DW
n=2
n=2
Interstitial Fluid Presssure, mm Hg


A
Interstitial Fluid Volume, ml/g DW
0.5
1
1.5
2
-2
-4
-6
-8
-10
-12
Interstitial Fluid Presssure, mm Hg
n=8
n=8
n=2
n=2
B
n=8
n=8
0.5
1
1.5
2
0
n=2
n=2
Interstitial Fluid Volume, ml/g DW
-2
-4
-6
-8
-10
Interstitial Fluid Presssure, mm Hg


A
3.5
3
2.5
2
1.5
1
GAG radius, same scale as y
n=8
n=8
n=2
n=2
1
1.5
2
2.5
3
3.5
4
ECM cell half thickness y, unit on nm or μm scale
B
n=8
0.5
1
1.5
2
2.5
3
3.5
4
0
-2
-4
-6
-8
Interstitial Fluid Presssure, mm Hg
n=8
n=2
n=2
Interstitial
Fluid Volume,
ml/g DW


A
ECM
ECM
ECM
2y
GAG
GAG
GAG


B
Collagen variations u along ±y
ECM
ECM
ECM
Fixed - ends
Free - ends