Compressibility of biological systems: The viscoelastic Poisson's ratio

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Abstract

Soft tissues carry out their vital biological functions within a dynamic mechanical framework that can be extended or compressed. Externally or internally-applied uni-axial or biaxial changes induce longitudinal strains that can be of either sign. The complex interrelationship between applied strain and induced strain is quantified by a time-space change of the Poisson's ratio, which is itself determined by cell-cell and cell-matrix interactions. While the viscoelasticity of multicellular systems under various experimental conditions has already been discussed extensively, the role of the viscoelastic Poisson's ratio, as a vital indicator of tissue compressibility, is only now beginning to be appreciated and explored more thoroughly. Tissues have frequently been treated as incompressible. However, the porous structure of the cell membranes, tissues, and extracellular matrices ensures an outflow of liquid even under relatively modest physiological strain conditions.

This study explores a range of tissues and biological composites consisting of multiple cell types and extracellular matrices in the context of compressibility, accompanied by their Poisson's ratio. They are subjected to strains induced by both external and internal factors that mimic physiological conditions.

Key words: viscoelasticity, collective cell migration, tissue fragility, semi-flexible filaments, cell-cell interactions

1. Introduction

Biological soft tissues engage in their essential functions within a dynamic mechanical landscape characterized by heightened levels of stretching at both the cellular and tissue levels [1-3]. For example, during epithelial morphogenesis, cell populations undergo considerable modifications in their surface area and volume, which is crucial for the development of complex three-dimensional configurations [4]. Various physiological processes, including the actions of breathing, the pulsations of the heart, peristaltic movements, and collective cell migration lead to the extension of epithelial tissues in multiple organs [5]. Endothelial cells are continuously subject to mechanical forces resulting from blood circulation due to the rhythmic contractions of the heart. The stretching induced by these forces leads to a range of effects that can be either advantageous or detrimental, contingent upon whether the mechanical stresses remain within physiological limits or surpass them [3]. The physiological extensional strain lies in the range of 5-15% [3]. Longitudinal uni-axial (or biaxial) extension induces transverse contraction (or extension) strain, which impacts on the volume of a multicellular system [4]. These structural changes can be discussed in the context of the Poisson's ratio.

Poisson's ratio is a physical parameter representing the ratio between the induced transverse contraction (or extension) strain to the applied longitudinal uni-axial (or biaxial) extension (or contraction) strain in the direction of applied force for 3D systems. Otherwise, it is the ratio between the strain that is induced perpendicularly to the externally/internally applied strain. This parameter can be used as a measure of the system compressibility under strain conditions and is closely connected with cell-cell and cell-matrix interactions. The difference between the Poisson's ratios obtained by uni-axial stretching/compression of a soft matter system in different directions can be an indicator of anisotropic behaviour. In homogeneous and isotropic soft matter systems, the relationship between various mechanical moduli such as the Young's modulus, the shear modulus, and the bulk modulus can be established, based on the Poisson's ratio [6]. For viscoelastic systems, the induced longitudinal strain is capable of relaxing under applied strain. Consequently, the viscoelastic Poisson's ratio is time-dependent and has been discussed in terms of a distribution of delay times [6]. The magnitude, rate, and direction of deformation all play crucial roles in determining the viscoelastic Poisson's ratio. Consequently, the viscoelastic Poisson's ratio can be considered in both time and frequency modes [7]. The complex Poisson's ratio, obtained in the frequency-mode, possesses real and imaginary parts. While the real part represents Poisson's ratio, the imaginary part quantifies the transverse strain lag caused by the damping effects of structural changes [7]. Boiko et al. [8] proposed an experimental technique for measurement the complex Poisson's ratio based on forced vibration responses for the samples under normal and shear deformation by varying amplitude ratio and phase lag of the forcing and response oscillations.

The estimation of Poisson's ratio offers a valuable opportunity for characterising inhomogeneous and anisotropic viscoelastic solids, such as biological tissues. Despite the importance of Poisson's ratio as an indicator of cell rearrangement under various experimental conditions, this physical parameter is only starting to be elucidated. Many experimental and theoretical papers assume the incompressibility of multicellular systems by setting Poisson's ratio to a value of 0.5. However, this assumption is rarely justified in the case of human tissues [9]. It is well known that biological tissues generally possess a porous structure [10]. The main reason for effectively neglecting the volumetric effects of cell rearrangement under strain conditions is the difficulty of measuring Poisson's ratio [9]. In most of the medical elasticity imaging techniques described in the literature, mechanical moduli of the tissue are reconstructed using two fundamental assumptions: (i) that the tissue behaves as a

perfectly linearly elastic solid, and (ii) that the tissue is incompressible or nearly incompressible [5,9,11]. This latter assumption could be acceptable, but only under very low strain. However, various experiments have pointed to the reality that soft tissues are in fact compressible, even under physiological conditions [9,12,13]. Some authors have provided an effective (averaged) value of Poisson's ratio, rather than its space-time distribution, in characterising biological tissues and other viscoelastic, inhomogeneous, and anisotropic materials.

Biological tissues, such as connective tissues, are exposure to induced strain conditions under physiological conditions. Blood flow induces oscillatory volumetric strain and shear strain of blood vessels. Collective cell migration, as an integral part of morphogenesis, wound healing, and spreading of cancer, induces self-generation of uni-axial and/or biaxial longitudinal strain. It is of crucial importance to be able to predict or measure the magnitude of the induced transverse strain within 2D and 3D multicellular systems in the form of the Poisson's ratio. This transverse strain can lead to: (i) disorder of collagen fibers and (ii) perturbation of cell-cell and cell-matrix interactions. Although the general features of the viscoelasticity of multicellular systems have been discussed extensively, Poisson's effect has received less attention.

Variations in Poisson's ratio within biological tissues can serve as an early indicator of emerging pathological conditions. This physical parameter can exhibit changes with the progression of various diseases [9], yielding information that is potentially valuable in clinical settings. Understanding Poisson's ratio in conjunction with mechanical moduli has demonstrated significant clinical relevance for the diagnosis, prognosis, and treatment of cancers. It plays a crucial role in quantifying tissue structural parameters, including vascular and interstitial permeability, both of which hold substantial clinical importance [1]. Hyper-permeability is linked to a range of diseases, including intestinal disorders, atherosclerosis, and thrombosis, among others [3,14,15]. Consideration of Poisson's ratio is relatively new and can be measured by electrography techniques under in vivo conditions [1].

This review primarily aims to elucidate the key physical factors that affect the viscoelastic Poisson's ratio in biological tissues. We will examine a range of multicellular systems and biological composites, that are composed of diverse cell types and extracellular matrices, while subjected to both externally and internally-induced strains that reflect physiological conditions. The systems under consideration include: (i) endothelial monolayers experiencing externally applied uni-axial extensional strain, (ii) the collective migration of epithelial monolayers across substrate matrices, (iii) the fusion process of two cell aggregates, (iv) the compression of cancer spheroids encased in extracellular matrix, (v) collagen I networks subjected to externally induced uni-axial extensional strain, (vi) skin, and (vii) blood vessels.

2. Viscoelastic Poisson's ratio: theoretical consideration

Poisson's ratio is defined as the ratio between the transverse contraction (or expansion) strain to the longitudinal uni-axial or biaxial extension/compression strain in the direction of the applied force, as shown schematically in **Figure 1**.

Figure 1.

This parameter can be used as a measure of the system compressibility under strain conditions. The volumetric strain of a 3D soft matter cuboid can be expressed as:

$$\varepsilon_V(t) = (1+\varepsilon_{xx})\big(1+\varepsilon_{yy}\big)(1+\varepsilon_{zz}) - 1 \qquad (1)$$

where $\varepsilon_V(t) = \frac{dV(t)}{V_0}$ is the volumetric strain, V_0 is the initial volume, and ε_{xx}, ε_{yy} and ε_{zz} are the diagonal components of the strain tensor. For the uni-axial extension in the *x*-direction, the longitudinal strain is $\varepsilon_{xx} > 0$, while the transverse strains ε_{yy} and ε_{zz} can be larger than zero for extension or less than zero for compression. Changes of the longitudinal strain components in the y- and z-directions of a viscoelastic solid under instantaneously induced uni-axial transverse elongation strain ε_{xx0} can be expressed as: $\varepsilon_{yy}(t) = -\nu_{xy}(t)\varepsilon_{xx0}$ and $\varepsilon_{zz}(t) = -\nu_{xz}(t)\varepsilon_{xx0}$ (where ε_{yy} and ε_{zz} are the components of volumetric strain in the y- and z-directions, respectively, while $\nu_{xy}(t)$ and $\nu_{xz}(t)$ are the corresponding viscoelastic Poisson's ratios).

These transverse strain components are $\varepsilon_{yy} = -\nu_{xy}\varepsilon_{xx}$ and $\varepsilon_{zz} = -\nu_{xz}\varepsilon_{xx}$. When longitudinal extension induces transverse contraction, the Poisson's ratio ν is positive, i.e., $\nu > 0$. In this case, a system's volume can: (i) increase for $\nu < 0.5$, (ii) stay constant for $\nu = 0.5$, or (iii) decrease for $\nu > 0.5$. However, when longitudinal extension induces transverse extension, which is a characteristic of auxetic materials, the Poisson's ratio is negative, i.e. $\nu < 0$, and the volume increases. In the case of 2D systems, the Poisson's ratio can be used as an indicator of the surface change under externally/internally applied uni-axial strain.

The Poisson's ratio depends on the magnitude, rate, and direction of stretching/compression. Under low strain conditions, some soft matter systems can behave as isotropic, elastic solids, while higher strain can induce an anisotropic, viscoelastic response. Higher strain rates can generate plastic, rather than viscoelastic, longitudinal strain. The viscoelastic Poisson's ratio is time-dependent.

Change of longitudinal strain simultaneously with changing uni-axial transverse strain is a characteristic of creep flow under constant stress, i.e. $\nu(t) = \frac{\varepsilon_t(t)}{\varepsilon_l(t)}$, and can be expressed as:

$$\varepsilon_t(t) = -\nu_0\varepsilon_l(t) + \int_0^t \nu(t-t')\frac{d\varepsilon_l(t')}{dt'}dt' \qquad (2)$$

where $\varepsilon_t(t)$ is the transverse strain, $\varepsilon_l(t)$ is the longitudinal strain, ν_0 is the initial Poisson's ratio, and $\nu(t)$ is the viscoelastic Poisson's ratio. The Poisson's ratio in experiments on stress relaxation under step longitudinal strain expressed as: $\varepsilon_l(t) = \varepsilon_0 H(t)$ is equal to $\nu_R(t) = \frac{\varepsilon_t(t)}{\varepsilon_0}$ (where $\nu_R(t)$ is Poisson's ratio in relaxation, $H(t)$ is the Heaviside step function at time t, and ε_0 is a constant longitudinal strain for $t > 0$) [16].

The corresponding pseudo-constitutive $\varepsilon_t(t)$-$\varepsilon_l(t)$ model for linear viscoelasticity could be expressed as: $\sum_{i=0}^{N} a_n \frac{d^n\varepsilon_l}{dt^n} = \sum_{i=0}^{N} b_n \frac{d^n\varepsilon_t}{dt^n}$ (where a_n and b_n are model parameters and N is the number of harmonics) [7]. Altered system structural changes prompted by the application of longitudinal strain, can lead to unexpected variations in the transverse strain. These variations are associated with anomalous diffusion processes, specifically sub-diffusion and super-diffusion, which characterize the movement of the system's components. In this context, the correlation between longitudinal and transverse strain can be articulated through fractional constitutive models [17,18]. Yin et al. [17] examined the fractional Maxwell model, the Kelvin-Voigt model, and the Zener model. The constitutive model could be transformed from the time domain into the frequency domain using the Fourier integral transform. The transformation equation may be expressed as $F[\varepsilon_l(t)] = -\nu^*(\omega)F[\varepsilon_t(t)]$ where $F[\cdot]$ is the Fourier transform, ω is the angular velocity, and $\nu^*(\omega)$ is the complex Poisson's ratio $\nu^*(\omega) = \nu'(\omega) + j\,\nu''(\omega)$ (where $\nu'(\omega)$ and $\nu''(\omega)$ are its real and imaginary parts and $j = \sqrt{-1}$ is the imaginary unit) [7, 16]. The real part $\nu'(\omega)$ is known as the dynamic Poisson's ratio, while the imaginary part $\nu''(\omega)$ is related to the strain lag caused by damping in the material

structural changes [7]. The ratio of the imaginary part to the real part of the complex Poisson's ratio is named Poisson's loss factor [7]. The corresponding Poisson's ratio in the time-domain has been expressed by Tschoegl et al. [6] as:

$$\nu(t) = \nu_{eq} - \sum_{i=0}^{N} \nu_i \, e^{-\frac{t}{\tau_i}} \qquad (3)$$

where ν_{eq} is the equilibrium Poisson's ratio, and ν_i are harmonics connected to various relaxation times τ_i. Shorter relaxation times quantify structural changes of smaller constitutive parts excited under lower longitudinal strain. However, higher induced strain can result in the cumulative excitation of larger structural parts. In this case, instantaneously induced uni-axial transverse elongation strain ε_{xx0} (with all parameters from eq.3 such as ν_{eq}, N, and ν_i, depends on the induced transverse strain ε_{xx0}. Having thus introduced fundamental aspects of the viscoelastic Poisson's ratio, we will now use it as the context within which to discuss a variety of biological systems.

Given that the dynamic Poisson's ratio depends on the magnitude, rate, and direction of stretching/compression, we will discuss relevant experiments in terms of cell rearrangement.

3. Compressibility of multicellular systems under various strain conditions applied externally/internally

Physiological mechanisms, including respiration, cardiac pulsations, collective cell migration, and tumour development caused by cell division result in oscillatory change in strain that manifest over different temporal scales. Respiration operates on a time scale of seconds, whereas heartbeats occur within a range of milliseconds to seconds. In comparison, the changes in strain related to collective cell migration are evident over a time frame of hours, whereas the cell divisions that contribute to tumour development occur on a time scale ranging from hours to days, contingent upon the specific cell type involved.

Choi et al. [3] considered the instantaneous and delayed responses of HUVEC endothelial monolayers, adhered onto a deformable elastomer layer, for high rate uni-axial extension in the range of 3-45%. Physiological cyclic extension corresponds to 5-15%, while in pathological extension the strain can exceed 20 %. Uni-axial extension causes transverse contraction strain of both the monolayer and substrate matrix. The resultant equilibrium Poisson's ratio, representing a combination of the rheological behavioura of the cell monolayer and the matrix is within the range of $\nu = 0.3$ for higher values of applied uni-axial extension strain to $\nu = 0.5$ for lower values. Higher strains $\geq 35\%$ accompanied by high strain rate cause the formation of small fractures, leading to an increase in the monolayer surface area. These fractures healed again after 30min of the extension. Lower strains ~ 8 % did not result in fractures and, in this case the surface area remained approximately constant. Such fractures influence the permeability of blood vessels. Leiderman et al. [1] also pointed out that Poisson's ratio can be used as an indicator of the permeability of blood vessels.

Moisdon et al. [12] measured the Poisson's ratio of Madin-Darby Canine Kidney-MDCK and HeLa epithelial monolayers on a flat substrate in frequency-mode for the frequency range $10^{-2} - 10^{2}$ Hz, which corresponds to a structural change in the range of milliseconds to minutes. The Piosson's ratio is frequency independent and equal to $\nu \sim 0.77$, indicating compression of the monolayers. A constant value of Poisson's ratio means that the transverse strain needs more time to relax under applied longitudinal strain. The strain relaxation time (i.e., the retardation time) under constant stress condition corresponds to a time scale of hours [19].

Physiological process such as directional collective cell migration, as an integral part of morphogenesis, wound healing, and the spreading of cancer, causes extensional and compressional strain [4]. Bhattacharya et al. [4] mapped the strain distribution during gastrulation of the zebrafish embryo and pointed out that some parts of the embryo undergo extension, while the others undergo compaction. The corresponding strain was in the range of ±20 %. The following scenarios, induced by cell signalling, were observed:

- The internal application of uni-axial extension results in perpendicular compression within certain multicellular regions.
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The distribution of the Poisson's ratio can be reconstructed from these experiments. It is found that its maximum value is $\nu \sim 1$, while in some regions of the embryo $\nu < 0$, pointing to auxetic behaviour, which is closely connected to the anisotropic nature of multicellular systems. This result demonstrated that a multicellular volume can increase or decrease and that cells are able to self-regulate the extent of such volumetric changes. Local changes in cell packing density significantly influence cell-cell interactions. Tlili et al. [20] revealed that the cell packing density varies from $1\text{x}10^{-3}$ to $4\text{x}10^{-3}$ $1/\mu\text{m}^2$ during collective migration of MDCK monolayers. Indirect indicators of the compressibility of epithelial monolayers, obtainable experimentally, include the generation of compressive stress during the rearrangement of confluent MDCK monolayers [21] and the local increase in cell packing density [20,22]. Migrating cell collectives show anisotropic behaviour caused by cell polarisation and orientation in the direction of movement, and they can induce an inhomogeneous accumulation of mechanical stress accompanied by increased cell packing density [20,21,23]. The maximum compressive stress generated during the rearrangement of confluent MDCK monolayers was 300Pa [21]. However, free expansion of MDCK monolayers generates tensional stress [23]. Consequently, internally generated uni-axial strain caused by collective migration of epithelial monolayers can result in either compressive or extensional strain components in the direction perpendicular to cell migration, as shown in **Figure 2**:

Figure 2.

Vercurysse et al. [24] pointed out that longitudinal extension leads to lateral compression of epithelial keratocyte 2D clusters, while the Poisson's ratio was not measured. Nevertheless, further experiments are required to gain a comprehensive understanding of the dynamics of collective cell migration and to delineate the distribution of Poisson's ratio along with its temporal variations. Oscillations of lateral strain, caused by collective migration of epithelial monolayers in the form of mechanical waves [21], can result in oscillations of Poisson's ratio as well. Compression of epithelial monolayers provokes various mechanisms of cell response such as: live cell extrusion and the cell jamming state transition [25]. Both processes are directed towards decreases in the induced compressive longitudinal strain and in Poisson's ratio.

The strain generation that occurs during collective cell migration within 3D multicellular systems can result in either system compression or system extension. A good example is the fusion of two cell aggregates under *in vitro* conditions, a process that deserves to be described in more detail. The fusion of cellular tissues is a widespread occurrence that offers significant technological opportunities,

particularly in the realm of tissue bio-fabrication. This process reflects very clearly the main differences between the collective movements of epithelial and cancer (mesenchymal) cells. Epithelial and cancer (mesenchymal) cells can perform various scenarios of cell rearrangement leading to either an increase or a decrease in the volume of a two-aggregate system. Two physical parameters in particular influence these scenarios: the compressive mechanical stress generated in the core region of the aggregates, and the surface tension between cell aggregates and the surrounding liquid medium [26]. Compressive stress has been shown to inhibit the motility of epithelial cells while simultaneously promoting the migration of cancerous mesenchymal cells [27,28]. Specifically, a compressive stress of 773Pa has been observed to decrease the movement of MCF-10A and MCF-7 cell lines [27]. This level of stress is representative of the compressive forces experienced within breast tissue due to cellular proliferation [29]. Normal mechanical stress, typically in the range of several hundred Pa, can arise from the collective dynamics of epithelial cell movement [21,30]. Conversely, this same stress can facilitate the enhanced motility of highly invasive cell lines such as 4T1 and MDA-MB-231, in addition to 67NR cells [27]. Surface tension can be responsible for the compaction of a two-aggregate system [31]. It is in accordance with the fact that the surface tension does work in reducing the surface area of a two-aggregate system by triggering collective cell migration. Surface tension depends on the strength of cell-cell adhesion contacts and cell contractility and varies from a few $\frac{\mathrm{mN}}{\mathrm{m}}$ to a several tens of $\frac{\mathrm{mN}}{\mathrm{m}}$ [32-34]. Epithelial cells establish strong E-cadherin-mediated cell-cell adhesion contacts, while cancer (mesenchymal) cells establish weak N-cadherin mediated adhesion contacts. Consequently, the epithelial surface tension is much higher than the cancer surface tension [35]. While extension of multicellular epithelial surfaces induces an increase in the surface tension, compression causes a decrease in the surface tension [31,36].

It means that epithelial cells migrate from the surface region of the two-aggregate system towards the core region driven by the epithelial surface tension, while the mechanical stress generated reduces their movement as shown schematically in **Figure 3a** [28,37].

Figure 3.

In contrast to epithelial cells, cancer cells migrate from the core region of cell aggregates towards to the surface region driven by the compressive stress accumulated within the aggregate core regions (**Figure 3b**). In both cases, collective cell migration induces an increase in the contact region between two cell aggregates. While epithelial cells perform volumetric rearrangement, cancer cells perform surface rearrangement (**Figure 3a,b**) [26]. The consequence of the volumetric rearrangement of epithelial aggregates is arrested coalescence caused by the cell jamming state transition as shown in **Figure 3a** [26,38]. Oriola et al. [39] identified effective viscosity, shear modulus, and surface tension as the primary physical parameters that contribute to the phenomenon of arrested coalescence. They characterized the viscoelastic properties of two-aggregate systems using the Kelvin-Voigt model. This arrested coalescence arises from positional and orientational interactions between cells, which are influenced by collective cell migration and growth [40]. In contrast to epithelial cells, cancer cells migrate along the surface of two aggregate systems and avoid the jamming [26,38]. Epithelial cells exhibit a tendency to form more spherical aggregates following complete coalescence, which is driven by the epithelial surface tension that reduces the surface area of the two-aggregate system (**Figure 3a**). In contrast, cancer cells tend to create irregularly shaped aggregates after total coalescence (**Figure 3b**). This observation aligns with the established fact that the surface tension of cancer (mesenchymal) cells is considerably lower than that of epithelial cells [35]. Dechristé et al. [41] investigated the fusion of two aggregates of human carcinoma cells (HCT116 cell line) due to cellular divisions during a 70-hour period. The doubling time for HCT116 cells is reported to be 18 hours [42].

However, this time can be prolonged in confluent surrounding such as core region of cell aggregates where majority of cells are arrested in G0/G1 phase of cell cycle [43]. Notable increases in both volume and surface area of the two-aggregate systems are observed within the initial 5 hours. During this early phase, changes in surface area and volume were primarily attributed to cell-cell interactions driven by the compressive stress that accumulates in the core region of the two-aggregate systems [26]. Specifically, the surface area of the two-aggregate systems expands by 1.8 times for larger aggregates (500 µm in diameter) and by 2.3 times for smaller aggregates (300µm in diameter) within the first 5 hours. In a related study, Shafiee et al. [44] examined the fusion of two confluent skin fibroblast cell aggregates, noting a decrease in surface area by a factor of 2.18 and a volume reduction of 2.38 times over a period of 140 hours. Change of the volume of two-aggregate systems could be quantified by their distribution of the Poisson's ratio. However, the local Poisson's ratio has not been measured in this type of experiment.

Alternative model systems that investigate volumetric changes resulting from cellular rearrangement, apart from the fusion of two cell aggregates, include the compression of cancer spheroids [14]. These model systems simulate tumour development within healthy tissue. The formation of tumour spheroids through collective cell migration and proliferation is limited by the surrounding microenvironment, which applies compressive stress. It would be intriguing to explore the extent to which cell aggregates can be compressed while maintaining the integrity of cell volumes under physiological conditions.

4. Externally induced compression of cancer spheroid surrounded by extracellular matrix

The expansion of a tumour is restricted by its surrounding microenvironment, which applies compressive stress; nevertheless, the pathways through which pressure disseminates within the tumour are not well understood. In a recent study, Dolega et al. [13] investigated the long-term structural changes in aggregates of CT26 mouse cells encased in an extracellular matrix under compressive stress by pointing out the volumetric effect of these changes. They were subjected to a compressive force resulting from an osmotic stress of 5kPa, which generated an equi-triaxial strain. The extracellular matrix (ECM), the major component of the tumour microenvironment, and its interactions with the tumour cells and stromal cells significantly influence cancer progression, from tumour initiation and growth to angiogenesis and invasion [45]. In this instance, the phenomenon of spheroid compaction cannot be analysed through the lens of Poisson's ratio, as all three components of volumetric strain are applied while none of them is induced.

The porous structures of extracellular matrices, multicellular systems, and single-cell membranes enable these systems to change their volumes under external mechanical or osmotic loading conditions [13]. The osmotic stress is generated through the application of: (i) big dextran molecules, characterized by a molecular weight of 500kDa and an average radius of gyration measuring 15nm and (ii) small dextran molecules, characterized by a molecular weight of less than 70kDa and an average radius of gyration measuring 5nm. Due to their substantial size, big molecules are unable to pass through the matrix. Instead, they promote an increase in the packing density of the cells, resulting in compressive stress, while the overall volume of individual cells remains relatively constant. In contrast to large molecules, small molecules can pass through the membrane towards the cells causing a decrease in single-cell volumes, but they cannot penetrate into individual cells.

An osmotic stress of 5kPa, caused by large dextran molecules, induces cell aggregate compression resulting in a decrease in the aggregate volume of 17%, while the single-cell volumes remained approximately constant, as shown in **Figure 4a**.

Figure 4.

This stress supresses cell division in the core region of spheroids [46]. The altered increase in cell packing density is related to: (i) cell shape change; (ii) cell orientation; and (iii) a decrease in the cell-cell separation. Selective compression, caused by small dextran molecules, deforms cells, while the cell packing density stays approximately constant [13]. This altered compression of the single-cells decreases the single cell volume by ~15% while cells maintain their activity (**Figure 4b**).

Mokbel et al. [47] measured the Poisson's ratio of the actin cytoskeleton caused by uni-axial compression of a single HeLa cell. The rearrangement of the actin cytoskeleton, similarly to a collagen I network, shows anisotropic and inhomogeneous behaviour. Actin filaments have a persistence length L_p of $\sim 18\ \mu m$, similar to their typical contour length in cells, classifying actin filaments as semi-flexible polymers [48]. The experimental Poisson's ratio of ~ 0.66 for the frequency corresponding to single-cell contractions [47]. This result confirms the compressibility of the actin cytoskeleton. An increase in frequency decreases the Poisson's ratio due to the reduced mobility of the cytoskeletal filaments. Generation of cell contractile stress perpendicular to the direction of cell migration discussed by Nejad et al. [49] can be caused by cell lateral compaction caused by cell oscillatory longitudinal uni-axial extension.

The Poisson's ratio of some biological composites such as skin and blood vessels represent a product of the integral contributions of the various layers. It would be interesting to see which layer exerts the dominant influence on Poisson's ratio in these cases.

5. Poisson's ratio of biological composites

The skin has three layers—the epithelial layer (epidermis), dermis, and hypodermis. The dermis has connective tissue, blood vessels, oil and sweat glands, nerves, hair follicles, and other structures. The hypodermis consists of connective tissue and adipose tissue. Blood vessels are made also of three layers. The outer layer, made by connective tissue, provides structural support and shape to the vessel. The middle layer composed of elastic and muscular tissue, which regulates the internal diameter of the vessel. The internal layer consists of a monolayer of endothelial cells. In some cases, connective tissue contributes significantly to the resultant values of Poisson's ratio for these biological composites [50]. Connective tissue typically features a disorganized three-dimensional or nearly two-dimensional (2D) network of collagen fibers. As reported by Hulmes [50], the dry weight composition of the cruciate ligament consists of 78% collagen, while the meniscus has a significantly higher collagen percentage of 90%. Additionally, the subcutaneous part of the skin is composed of 65% collagen, and tendon reflects a collagen content of 78%. Consequently, we will discuss the main properties of the Poisson's ratio of collagen networks.

5.1 Poisson's ration of collagen I networks

Collagen fibers are semi-flexible and form anisotropic networks. The main condition of semi-flexible filaments is that the filament contour length L_c is of approximately the same order of magnitude as the persistent length L_p. The persistence length of the collagen fibers varies from $L_p \sim 14 - 180\text{nm}$,

while the contour length is $L_c \sim 309\text{nm}$ [51]. The viscoelasticity and pore size distribution of physically cross-linked collagen I networks depend on the collagen concentration, length of collagen fibers, and crosslinking density, as well as on the direction of the external strain with respect to fiber orientation [51-54]. Consequently, the rearrangement of collagen fibers significantly influences the rheological behaviour of connective tissues and hence their Poisson's ratio. Uni-axial extension in the direction of fiber orientation leads to network compression due to a transition from conformational changes of fibers related to entropic effects, to filament stretching related to enthalpic effects [55]. Stretching of semi-flexible filaments has been described by a worm-like chain model expressed in terms of the stretching force vs. filament extension [56]:

$$F_{wlc} = \frac{k_B T}{L_p}\left[\frac{1}{4(1-x)^2} - \frac{1}{4} + x\right] \quad (3)$$

where F_{wlc} is the stretching force, k_B is Boltzmann constant, T is temperature, x is the stretch ratio expressed as $x = \frac{R - \langle r_g^2 \rangle^{1/2}}{L_c}$, R is the filament end-to-end distance, and $\langle r_g^2 \rangle^{1/2}$ is the average filament radius of gyration. While the force-stretch ratio relationship is linear for induced conformational changes of the filament for $x \ll 1$, this relationship becomes non-linear for the case of filament stretching when $x > 1$.

The equilibrium Poisson's ratio corresponding to a 20% uniaxial extension of collagen I networks, aligned with the orientation of the fibers, is approximately $\nu \sim 5$, which is attributable to the compression of the network [57]. Longer fibers accompanied by larger pore sizes lead to higher Poisson's ratio under the same uniaxial stretching and concentration of fibers [57]. The latter authors apparently demonstrated that a lower concentration of collagen corresponds to a higher Poisson's ratio, whereas Picu et al. [58] reported findings that contradict this conclusion. The difference could be caused by a different orientation of the uni-axial stain relative to the average fiber orientation within the network in these experimental setups.

5.2 The Poisson's ratio of skin

The hypodermis, composed of collagen matrix, significantly influences the Poisson's ratio of skin. Picu et al. [58] considered changes in the incremental Poisson's ratio $\nu^{inc} = -\frac{d\varepsilon_t}{d\varepsilon_l}$ under uni-axial stretching of human amnoion, mouse skin, and collagen gel in the direction of the preferential fiber orientation. The incremental Poisson's ratio increased with the extensional strain up to 35% and reached a maximum value of $\nu^{inc} > 3$ for all examined systems, pointing to intensive compressibility. Extensional strain higher than 60% leads to the generation of a plastic strain contribution within the skin [59]. Below this strain, the skin behaves viscoelasticaly. Uni-axial extension in the direction perpendicular to the oriented fibers can induce auxetic behaviour caused by a disordering of collagen fibers.

Dwivedi et al. [60] considered changes in the Poisson's ratios ν_{xy} and ν_{xz} of porcine skin caused by a 20 % uni-axial extension parallel/perpendicular to skin tension lines. The parameter ν_{xy} increased from 0.423±0.053 to 1.130±0.210, while the parameter ν_{xz} increased from 0.614±0.102 to 1.381±0.351 for stretching parallel to skin tension lines. This emphasises the intensive compression quantified by the resultant compressive strain of -33%. However, stretching perpendicular to skin tension lines led to auxetic behaviour resulting in a volume increase. In this case, ν_{xy} changed from 0.441±0.038 to −1.710±0.290, while ν_{xz} increased from 0.521±0.156 to 1.411±0.289, quantified by the resultant extensional strain of 15%. The skin sample could be treated as incompressible for lower uni-axial

extension perpendicular to skin tension lines, which is not able to induce breaking of inter-filament bonds.

5.3 The Poisson's ratio of blood vessels

As described above, blood vessels are made up of three layers: an outer layer of connective tissue; a middle layer of elastic, muscular tissue; and an internal lining of endothelial cells. The latter are exposed to the shear stress caused by blood flow. It is the elastic muscular tissue, rather than the connective tissue, that influences the rheological behaviour of blood vessels. This is consistent with the fact that the arterial wall is commonly considered as incompressible.

Carew et al. [61] pointed out that the volumetric strain of arterial wall under physiological conditions is $\sim 1.65\%$. The in-plane Poisson's ratio of arterial wall was assessed as being in the range of 0.3-0.4, whereas its out-of-plane component is much higher, ranging from 0.5-0.7, which indicates the anisotropic behaviour of arterial structural changes under mechanical stress caused by blood flow [62]. Hasegawa et al. [63] proposed a non-invasive ultrasound technique to measure experimentally the Poisson's ratio of the carotid artery and abdominal aorta. Their method employed a high spatial resolution technique to measure them in both the axial and radial directions of the arterial wall, leading to the conclusion that the Poisson's ratio of the arterial wall was 0.46.

Cells are sensitive to slight variations in both the magnitude and direction of blood flow-induced shear stress, as well as to perturbations in this stress [64]. Alterations in shear stress can impact cell morphology, blood vessel permeability, and cell-cell adhesion contacts, and they may trigger inflammatory responses. Higher or perturbed shear stress has the potential to initiate the development of vascular conditions like atherosclerosis and aneurysms [65]. Cells are able to regulate the shear stress generated by blood flow to a certain degree by collective cell migration, by changing the strength of cell-cell adhesion contacts and through cell shape changes [66]. Migration of cells in the direction of blood flow decreases the shear stress, while cell migration against the flow increases the shear stress. Changes in cell shape and orientation influence the diameter of blood vessels and consequently alter the shear stress as well [67]. These structural changes have an impact on the rheology of blood vessels and on the Poisson's number as well. A decrease in the Poisson's ratio can serve as an indicator of an increased permeability of blood vessels.

6. Conclusion

We have focused on the role of the Poisson's ratio as an indicator of tissue compressibility, in cell rearrangement under externally and internally applied strains that reflect physiological and pathological conditions. Soft tissues such as epithelial and endothelial multicellular systems behave as inhomogeneous and anisotropic viscoelastic solids. Biological composites often possess a multi-layered architecture and are comprised of a diversity of cell types along with extracellular matrix components, all of which play a role in the viscoelastic properties of these intricate systems. Such systems exhibit sensitivity to the direction, magnitude, and rate of change of applied strains, whether external or internal.

Poisson's ratio characterizes the relationship between the transverse strain, whether uni-axial or biaxial, that is applied externally or internally, and the resulting longitudinal strain. The relaxation of longitudinal strain, which is influenced by interactions between cells, may result in the development of voids. This increases the volume of endothelial monolayers and enhances the porosity of blood

vessels. In this context, an increase in volume can be represented by a reduction in the Poisson's ratio. Collective cell migration, a fundamental process occurring during morphogenesis, wound healing, and the dissemination of cancer through epithelial tissues, can induce both scenarios: localized compression or extension within multicellular epithelial structures. This compressive longitudinal strain may initiate a transition to a cell-jamming state within epithelial systems, or can facilitate the extrusion of live cells. Both phenomena aim to reduce the compressive longitudinal strain, which is associated with changes in the Poisson's ratio. Otherwise, extensional longitudinal strain leads to an increase in the epithelial surface tension. Further experimental investigations are required to elucidate the distribution of the Poisson's ratio and the alterations induced by collective cell migration. It will be essential to relate the alteration of Poisson's ratio to the interactions between cells and between cells and the extracellular matrix at both cellular and subcellular scales.

The anticipated compaction of tumour's spheroids throughout their development, resulting from interactions with adjacent healthy tissue, may exceed 15%. In such instances, the cells maintain both their volume and functional activity.

The viscoelastic properties of the extracellular matrix and their influence on the rheological behavior of various biological composites, including skin and blood vessels, were examined through the lens of Poisson's ratio. The rheological characteristics of the extracellular matrix, primarily composed of collagen I, under uniaxial strain conditions, play a crucial role in determining both the magnitude and variation of Poisson's ratio. Its value is $\nu > 0.5$, pointing to skin compression for uni-axial extension applied parallel to skin tension lines. However, when uni-axial extension is applied perpendicular to skin tension lines, skin behaves as an auxetic material, i.e. the corresponding Poisson's ratio is $\nu < 0$ as an indicator of intensive increase in the skin volume. In contrast to the rheological properties of skin, the extracellular matrix does not significantly affect the rheological behaviour of blood vessels, which behave as incompressible materials under physiological conditions.

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Figure captions:

Figure 1. Schematic representation of Poisson's ratio.

Figure 2. Local extension/compression caused by collective cell migration of epithelial monolayers on a substrate matrix. Blue and green arrows represent the directions of longitudinal and transverse strain components.

Figure 3. (a) Volumetric rearrangement of epithelial cells and (b) surface rearrangement of cancer (mesenchymal) cells caused by collective cell migration during the fusion of two cell aggregates. Blue arrows represent a direction of cell movement. The cell aggregate formed through the total coalescence of epithelial cell aggregates exhibits a more spherical shape compared to that resulting from the coalescence of cancer cell aggregates. The primary physical factors contributing to these variations are the surface tensions associated with epithelial and cancer cells.

Figure 4. Compression of cancer spheroids under the same osmotic stress produced by: (a) big dextran molecules; and (b) small dextran molecules. The blue arrows point to the fact that big dextran molecules induce compression of both extracellular matrix and cell aggregates. The green arrows point to the fact that only the cells are compressed, leading to an extension of the extracellular matrix.