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SENSITIVITY OF DONNAN'S MODEL PARAMETERS TO THE MODELING OF OSMOTIC PRESSURE IN TENDONS

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Abstract. *Tendons are fibrous connective tissues that contain a significant amount of water in their composition. However, the specific role of water in mechanical, chemical and biological functions has not been clearly stated in the literature, requiring further research. In this context, the aim of this study is to investigate the extent to which the parameters of the well-known Donnan's model affect the overall osmotic pressure within tendon tissues. These parameters include temperature, fluid volume fraction, external chemical concentration and fixed charge density (FCD). In order to establish reference values for each of these parameters, a literature survey and a series of in vitro experiments were performed. Firstly, a range of temperatures that tendons can experience were obtained from previously published in vivo experiments. Secondly, the fluid volume fraction was characterized using a water content protocol combined with a digital image segmentation approach. Thirdly, the external chemical concentration was measured from a specific polyethylene glycol buffer solution, designed to mimic the in vivo chemical environment of tendons. Lastly, the fixed charge density was estimated based on the total sulfated glycosaminoglycans (GAG) content, which was quantified through a biochemical assay (Dimethylmethylene Blue Assay – DMMB Assay). All in vitro experiments were performed on bovine tendons. The achieved results are preliminary in nature and are intended to contribute to future research in this field using numerical simulations based on biphasic-swelling models.*

Keywords: *Tendon, Osmosis, Interstitial fluid, Poroelastic, Donnan's model*

1. INTRODUCTION

Tendons are fibrous connective tissues that contain a significant amount of water, approximately 50-75% of their composition (Lozano et al. 2019; Mlyniec et al. 2021). The dynamics of water in tendons are believed to play a crucial role in their biomechanics, controlling tissue homeostasis through mechanotransduction pathways, cell nutrition, among other functions (Hunckler et al. 2015; Khayyeri et al. 2015; Lavagnino et al. 2015; Passini et al. 2021; Ristaniemi et al. 2021). However, the precise importance of water in mechanical, chemical and biological processes, as well as their interplays, remains unclear to this day.

In this context, computational models based on biphasic theories provide valuable tools for studying the dynamics of water in permeable (hydrated) solids, particularly in relation to interstitial fluids in biological tissues. These models typically assume that the material's microstructure consists of a homogeneous mixture of two phases: a *porous solid* phase and a *fluid* phase. While the former is usually modeled by an elastic or viscoelastic material, the latter is ruled by

the fluid pressure gradient, resulting in time-dependent behavior (Khayyeri et al. 2015; Ristaniemi et al. 2021; Klahr et al. 2022).

Within this theoretical framework, a specific class of models known as *biphasic-swelling* incorporates osmotic effects into the biphasic formulation. This is achieved by considering an osmotic pressure difference between the tissue's osmolarity and the chemical concentration of its external environment, using the concept of *water chemical potential* (Wilson et al. 2005; Galbusera et al. 2011). In this approach, the osmotic pressure is treated as a constitutive equation that is added to the stress of the solid phase, following a mixture model approach. Among the available options for osmotic pressure functions, those of Donnan's type is the most commonly used in the biomechanics field (Basilio et al. 2019; Nedrelow et al. 2021). Such models are usually characterized by four parameters: temperature, fluid volume fraction, external chemical concentration and fixed charge density (FCD).

Aiming future researches in this field by means of numerical simulation based on biphasic modeling, the present study concerns in investigating into what extent percentage variation on the aforementioned parameters affect the overall osmotic pressure that can occur within tendon tissues.

To establish reference values for these parameters, a literature survey and a series of *in vitro* experiments were carried out. The range of temperature variations that tendons can experience was obtained from *in vivo* experiments conducted elsewhere (Wilson and Goodship 1994). The fluid volume fraction was characterized using a water content protocol combined with a digital image segmentation approach (Carniel et al. 2023). The external chemical concentration was measured by an osmometer from a polyethylene glycol buffer solution (8%wt PEG-20 kDa with Tris-HCl). This solution aims to mimic the *in vivo* external osmotic environment of tendons and is capable of maintaining tissue hydration (Safa et al. 2017). Finally, the fixed charge density (FCD) was estimated based on the total sulfated glycosaminoglycans (GAG) content, which was quantified using a well-established biochemical assay (Dimethylmethylene Blue Assay – DMMB Assay), following a previously described protocol (Farndale et al. 1982; Bah et al. 2020). All the *in vitro* experiments were performed on *flexor digitorum profundus* bovine tendons.

2. MATERIALS AND METHODS

2.1 Theoretical background

Poromechanical models (also called biphasic models) have been commonly used to study the hydro-chemo-mechanical couplings of several biological tissues (Khayyeri et al. 2015; Ristaniemi et al. 2021; Klahr et al. 2022). Such models assume that the material microstructure is a homogeneous mixture of two phases: a *porous solid* phase (solid skeleton) and a *fluid* phase. The strong form of a biphasic problem can be written as follows:

$$\begin{cases} \operatorname{div}(\boldsymbol{\sigma}) = \mathbf{0} \\ \operatorname{div}(\mathbf{v}_s + \mathbf{w}) = 0 \end{cases} \tag{1}$$

In Eq. (1), the total Cauchy stress $\boldsymbol{\sigma} = \boldsymbol{\sigma}_s + \boldsymbol{\sigma}_f$ is given by the sum of the stress of the solid skeleton $\boldsymbol{\sigma}_s$ and the stress of the fluid phase $\boldsymbol{\sigma}_f = -p_f \mathbf{I}$, where p_f is the pore pressure. The vector $\mathbf{w} = v_f(\mathbf{v}_f - \mathbf{v}_s)$ defines a relative fluid velocity between the fluid and solid particles weighted by the fluid volume fraction v_f. In such models, a saturation assumption is generally employed, *i.e.*, $v_f + v_s = 1$, where v_s is the volume fraction of the solid skeleton. In addition, proper boundary conditions must be prescribed to the solid and fluid boundaries, and constitutive equations must be assigned for $\boldsymbol{\sigma}_s$ and $\mathbf{w}$ (Klahr et al. 2022).

In order to consider osmotic effects within the biphasic problem (1), an osmotic pressure difference $\Delta\pi$ between the tissue and the external solution must be taking into account. In this regard, the pore pressure can be redefined as

$$p_f = \mu_f + \Delta\pi \tag{2}$$

where μ_f is the so-called *water chemical potential* (Wilson et al. 2005; Galbusera et al. 2011). In view of Eq. (2), the total stress results in

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}'_s - \mu_f \mathbf{I}, \quad \boldsymbol{\sigma}'_s = \boldsymbol{\sigma}_s - \Delta\pi \mathbf{I} \tag{3}$$

where $\boldsymbol{\sigma}'_s$ plays the role of an effective stress of the solid skeleton in the sense of a mixture model.

This formulation is generally referred to as the *biphasic-swelling* model, and it is fulfilled with an appropriate constitutive equation for $\Delta\pi$. Among the possible choices of osmotic pressure functions, those of Donnan's type is the most used in the biomechanical field (Basilio et al. 2019; Nedrelow et al. 2021). From a phenomenological point of view, Donnan's osmotic pressure can be defined as

$$\Delta\pi = RT\left(\sqrt{C_F^2 + C_*^2} - C_*\right), \quad C_F = \frac{C_{F_0} v_{f_0}}{J - 1 + v_{f_0}} \tag{4}$$

in which $J = \det(\mathbf{F})$ is the volumetric Jacobian, $\mathbf{F}$ is the deformation gradient, R is the universal gas constant, T is the absolute temperature, C_* is the external chemical concentration and C_F is the fixed charge density (FCD), *i.e.*, tissue osmolarity. In such a model, the FCD is usually assumed to be a function of J controlled by its strain-free value C_{F_0} and bounded by the reference fluid volume fraction v_{f_0}.

The main goal of this study consists of a preliminary investigation of Eq. (4) by means of a sensitivity analysis of its parameters within a context of tendon tissues. In other words, it is investigated herein into what extent the osmotic pressure $\Delta\pi$ changes when prescribed variations on the quantities T, C_*, C_{F_0} and v_{f_0} are considered.

2.2 Experimental data

In this work, the sensitivity analysis on Donnan's parameters was performed using upper and lower bounds around referential values for the quantities T, v_{f_0}, C_* and C_{F_0}. The criteria used to define these values are detailed in the sequence.

The body temperature (37 °C) was chosen as the referential value for T. Since several experiments in tendons are performed in room temperature, the lower bound of 25 °C is investigated. Experimental works point out that the intratendinous core could reach temperatures up to 45°C during exercise (Wilson and Goodship 1994). Therefore, this value is set to the upper bound of T.

The referential fluid volume fraction was assessed following a protocol previously published (Carniel et al. 2023), given by

$$v_{f_0} = \frac{\left(m_f/\rho_f\right)}{V_t}\left[\frac{\mathrm{cm}^3}{\mathrm{cm}^3}\right] \tag{5}$$

where $\rho_f \approx 1\ \mathrm{g/cm^3}$ is the mass density of water, m_f is the mass of water of wet tissue samples in grams (water content) and V_t is the volume of wet tissue samples in cm^3. Briefly, samples with 5 mm length were cut from the mid-portion of *flexor digitorum profundus* bovine tendons (*bos taurus taurus*) and immersed for 12 h in 8%wt polyethylene glycol (PEG-20 kDa) prepared with Tris-HCl buffer (TBS, 10 mM, pH 7.6) in order to establish a basal osmotic pressure. This solution is capable to preserve the hydration of tendons (Safa et al. 2017). The mass m_f was directly assessed via a water content assay (drying procedure). The samples volume V_t, on the other hand, was characterized based on an image segmentation algorithm, where further details can be found elsewhere (Carniel et al. 2023). From these measurements, the mean value of $v_{f_0} = 0.78$ was calculated and assumed as the reference. Variations of ~10% around this reference were chosen to be the lower and upper values for v_{f_0}, *i.e.*, 0.70 and 0.86, respectively.

As previously mentioned, an 8%wt PEG-20 kDa with a Tris-HCl buffer is capable to mimic the external osmotic environment of tendons, being therefore, this solution used as reference for C_*. The solution osmolarity was measured in triplicate in a vapor pressure osmometer (VAPRO, Wescor EliTech), resulting in a mean of 170 mM. Variations of ~10% around this reference were set as the lower and upper values for C_*, *i.e.*, 153 mM and 187 mM, respectively.

In fibrous tissues, the fixed charge density (FCD) is mainly attributed to the negative charge character of the glycosaminoglycans (GAG), which in turn provide a hydrophilic behavior to the tissue and, therefore, controlling osmotic processes. Values of C_{F_0} have been estimated from biochemical essays (Shapiro et al. 2002; Oswald et al. 2008) by the formula

$$C_{F_0} = (GAG_{co}[\mathrm{mg/l}])\left(\frac{GAG_{cn}[-]}{GAG_{mw}[\mathrm{g/mol}]}\right) \quad [\mathrm{mM}] \tag{6}$$

In Eq. (6), the variable GAG_{co} represents the total sulfated GAG content (mass of GAG per volume of water within the wet tissue), which was quantified from the Dimethylmethylene Blue Assay – DMMB Assay (Farndale et al. 1982) performed in 5 samples of *flexor digitorum profundus* bovine tendons in duplicate, following a proper protocol for tendons (Bah et al. 2020). The variables $GAG_{cn} = 2$ and $GAG_{mw} = 463$ g/mol are the charge number and the molecular weight of the chondroitin sulfate (from shark cartilage), respectively, which was used as the standard in the biochemical assay. Along with the water content of 58% (Carniel et al. 2023), the FCD mean estimated by Eq. (6) results in $C_{F_0} = 23$ mM, which was set as the referential value. Variations of ~10% around this reference were chosen as the lower and upper values for C_{F_0}, *i.e.*, 20 mM and 25 mM, respectively.

All the chemicals used in this work were acquired from Merck (Sigma-Aldrich).

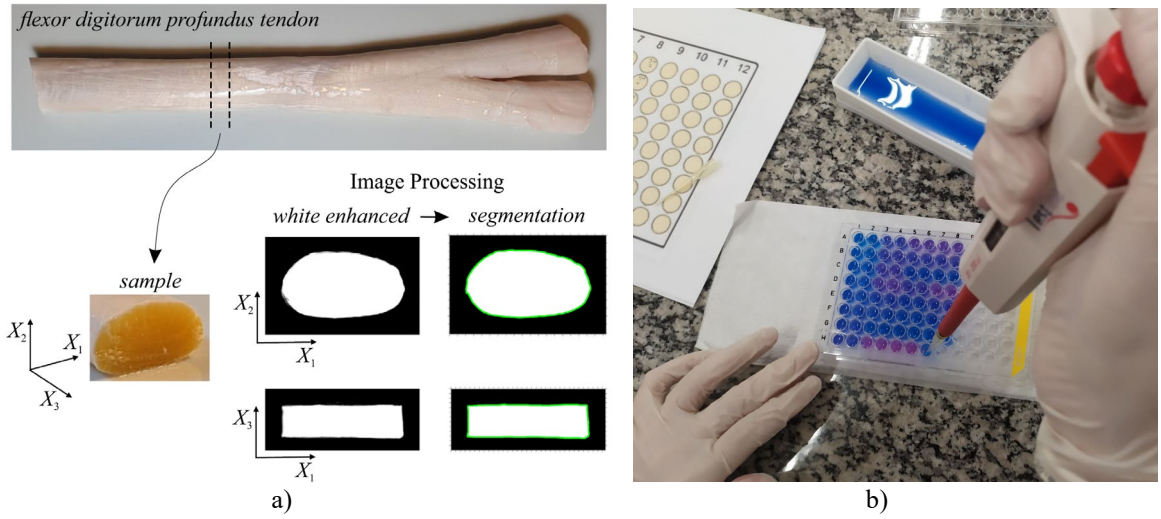

Figure 1. a) Samples with 5 mm length (X_3 direction) were obtained from *flexor digitorum profundus* bovine tendons. In order to better highlight the surface boundaries of the sample ($X_1 - X_2$ and $X_1 - X_3$ planes), the white character of the tissue was enhanced by adjusting the exposure time, contrast and brightness of the camera. Figure a) was obtained, with permission, from Carniel et al. (2023). b) Plate preparation for the DMMB assay used to quantify the GAG content within tendons.

3. RESULTS, DISCUSSION AND FINAL REMARKS

The results obtained from the sensitivity analysis on the parameters of Donnan's model are summarized in Figure 2. All curves are evaluated up to 20% volumetric deformations ($0.8 < J < 1.2$), since these values encompass the possible range of deformations that tendon tissues can experience (Carniel and Fancello 2017, 2019). All graphs of Figure 2 present the data in the same way: 1) the osmotic pressure predicted by Eq. (4) are plotted as solid lines and referenced to the left axes; 2) the sensitivity of the model response to its parameters is assessed by the difference percentage between the curves predicted by the reference values (ref.) and those of the variations. This is represented by the dashed lines and referenced to the right axes.

The influence of the temperature on Donnan's osmotic pressure is depicted in Figure 2a. It is observed that temperature acts as a scale parameter, shifting the curves in a constant manner and resulting in uniform percentage differences throughout the deformation process. This behavior is evident in Eq. (4), where the temperature is directly proportional to the pressure and independent of strain. Notably, for the variations evaluated around 37 °C, ~4% difference is achieved for 25 °C and ~2.5% for 45 °C, which are relatively small percentages. An interesting insight from these results suggests that osmotic experiments performed at room temperature may yield osmotic pressures with only slight deviations from those performed in a more precisely controlled temperature setup.

Figure 2b shows the results regarding the fluid volume fraction. It is evident that this parameter controls the osmotic pressure in a non-linear and nonuniform manner as function of the volumetric deformation, being more sensitive to compression. Regarding the sensitivity analysis, 10% variations around the reference value result in a maximum difference of ~4.5% under expansion and ~8% under compression. Moreover, small values of v_{f_0} lead to larger differences (compare the results for $v_{f_0} = 0.70$ and $v_{f_0} = 0.86$). Based on these findings, one can verify that the variation imposed (10%) and the maximum error achieved (~8%) are closely aligned, underscoring the need for careful treatment of v_{f_0} when employing *biphasic-swelling* models for tendon modeling. A comprehensive discussion on this topic can be found in Carniel et al. (2023).

The results regarding the external bath concentration are depicted in Figure 2c. Firstly, according to Eq. (4), although the variable C_* presents a nonlinear functional relation with osmotic pressure, the predicted curves show uniform percentage differences throughout the deformation process within the range of values analyzed. Secondly, a 10% variation around the reference value results in ~10% differences in the predicted curves for both expansion and compression. As previously mentioned, this one-to-one correspondence between variations and percentage differences emphasizes the significance of a prior knowledge of the chemical concentration of the external tendon environment.

Figure 2d addresses the results concerning the fixed charge density. Similar to what has been observed for the variable C_*, the strain-free value C_{F_0} of the FCD uniformly affects the percentage differences throughout the deformation process. However, in this case, the percentage difference values are significant, *i.e.*, ~17% for 25 mM and ~28% for 20 mM. Moreover, in this model, higher values of C_{F_0} correspond to higher osmotic pressures. Among the presented results, these last findings are particularly important. As can be seen, a 10% error in estimating C_{F_0} can result in an underestimation of the osmotic pressure by up to two times for lower values of C_{F_0} and an overestimation by up to three times for higher

values of C_{F_0}. Since, these large errors may significantly bias the results drawn by a *biphasic-swelling* modeling, well-designed experimental-numerical protocols should be proposed to accurately estimate the role FCD in tendons with respect to the model (4), for instance, by means of a fitting curve procedure (Carniel and Fancello 2017).

Finally, it is important to state that the present results are preliminary in nature and are intended to contribute to future research in this field using numerical simulations based on *biphasic-swelling* models.

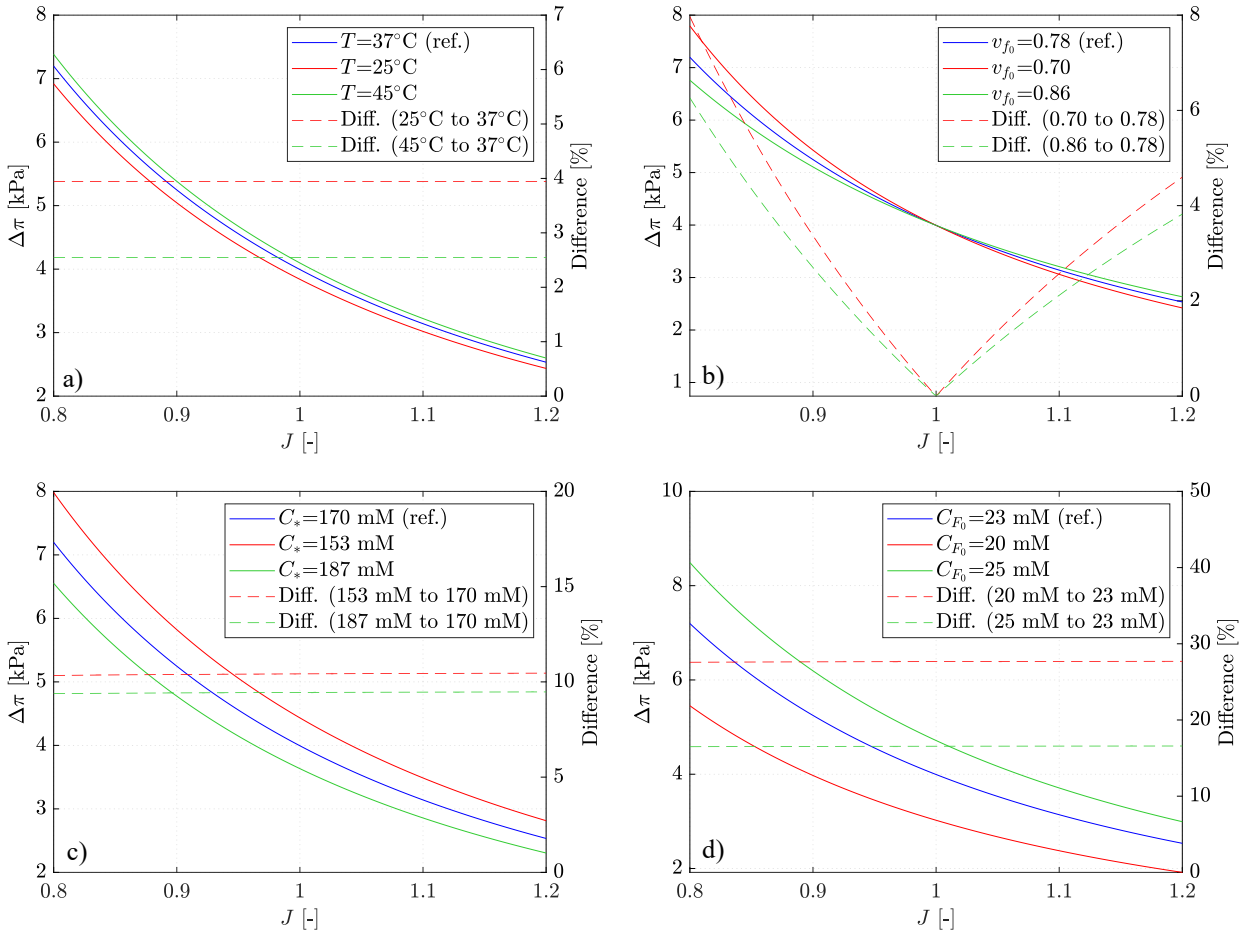

Figure 2. Osmotic pressures predicted by Donnan's model for variation around the reference values of the parameters (left axes, solid lines): a) temperature T; b) fluid volume fraction v_{f_0}; c) external chemical concentration C_*; d) fixed charge density C_{F_0}. The sensitivity of the model response regarding its parameters is assessed by the difference percentage between the curves predicted by the reference values (ref.) and those of the variations (right axes, dashed lines).

4. ACKNOWLEDGEMENTS

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