



## Effect of molecular weight and tissue layer on solute partitioning in the knee meniscus



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### ABSTRACT

**Objective:** Knee meniscus tissue is partly vascularized, meaning that nutrients must be transported through the extracellular matrix of the avascular portion to reach resident cells. Similarly, drugs used as therapeutic agents to treat meniscal pathologies rely on transport through the tissue. The driving force of diffusive transport is the gradient of concentration, which depends on molecular solubility. The meniscus is organized into a core region sandwiched between the tibial and femoral superficial layers. Structural differences exist across meniscal regions; therefore, regional differences in solubility are also hypothesized.

**Methods:** Samples from the core, tibial and femoral layers were obtained from 5 medial and 5 lateral porcine menisci. The partition coefficient ( $K$ ) of fluorescein, 3 kDa and 40 kDa dextrans in the layers of the meniscus was measured using an equilibration experiment. The effect of meniscal compartment, layer, and solute molecular weight on  $K$  was analyzed using a three-way ANOVA.

**Results:**  $K$  ranged from a high of  $\sim 2.9$  in fluorescein to a low of  $\sim 0.1$  in 40 kDa dextran and was inversely related to the solute molecular weight across all tissue regions. Tissue layer only had a significant effect on partitioning of 40k Dex solute, which was lower in the tibial surface layer relative to the core ( $p = 0.032$ ).

**Conclusion:** This study provides insight into depth-dependent partitioning in the meniscus, indicating the limiting effect of the meniscus superficial layer on solubility increases with solute molecular size. This illustrates how the surface layers could potentially reduce the effectiveness of drug delivery therapies incorporating large molecules ( $>40$  kDa).

### 1. Introduction

The meniscus fibrocartilage is crucial in maintaining knee function and protecting the neighboring tibial and femoral surfaces from excessive forces [1,2]. Degradation of the meniscus is associated with the initiation and progression of osteoarthritis (OA) in the knee [3]. Therefore, maintaining a healthy meniscus is essential for overall joint health. The development of new strategies for maintaining and/or restoring meniscus health relies upon a comprehensive understanding of the functional properties of the tissue and their relationship to meniscus structure and composition.

The delivery of nutrients within the meniscus is rather limited as vasculature is only found in the outer third of the tissue, referred to as the red zone. In contrast, the inner portion of the tissue is avascular; this is referred to as the white zone [4]. Accordingly, the white zone is not capable of self-repair due to the lack of direct blood supply [4–6]. Moreover, due to the avascularity of the white zone, resident cells must rely on transport from the outer region and surrounding synovial fluid to meet their metabolic needs (i.e., delivery of nutrients and removal of metabolic wastes) [7]. Therefore, studying the meniscus' transport properties is essential to understanding the tissue's potential healing response particularly with acute traumatic tears as well as meniscal degeneration.

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The composition and structure of meniscal tissue plays a key role in the transport of solutes through the tissue matrix [8]. For instance, tissue composition and structure is heterogeneous, varying between the inner core region and the outer superficial layers. In the core region of the tissue, the composition is approximately 75% water, 20% collagen fibers, and 5% non-collagenous substances including glycosaminoglycans (GAGs) [9]. Also, collagen fibers are oriented primarily in the circumferential direction with some aligned radially [10–12]. In contrast, the superficial layers have a lower GAG content and a more randomized collagen fiber organization [13–16]. The differing composition and structure may lead to regional differences in transport behavior.

The partition coefficient ( $K$ ) is an important transport property that describes the ratio of the concentration of a molecule within the tissue relative to the concentration in free solution at equilibrium [17–19]. When  $K$  equals unity, the solute is evenly distributed between the tissue and the bathing solution. However, when  $K < 1$ , this signifies that the solute is partially excluded from the tissue; this may be attributable to either steric hindrance (due to solute size) or because of repulsive electrostatic interactions between the solute and matrix. In contrast, when  $K > 1$ , this signifies an attractive electrostatic interaction between the solute and the matrix is present. Previous studies have shown that  $K$  in cartilaginous tissues depends on several parameters including the composition and structure of the tissue as well as the size and polarity of the solute [17,18,20–24]. In particular, a previous study on glucose partitioning in porcine meniscus showed that  $K$  is positively correlated with tissue water and GAG contents [17]. However, the effect of the specific tissue structure across meniscal layers on solute partitioning has yet to be examined. Moreover, while the partitioning of molecules across a range of sizes has been investigated in articular cartilage [20,24–26], there is no information about how size affects solute partitioning specifically in the meniscus.

The objective of the present study was to investigate the effect of solute molecular weight, meniscal layer, and meniscal compartment on  $K$ . Fluorescein (332 Da) was used as a size analog for nutrients, while fluorescence-labeled dextrans (3k and 40k Da) were used to represent larger molecules, such as growth factors and potential drug treatments for meniscal pathologies. We hypothesize that  $K$  is inversely related to the molecular weight of the solute. In addition, it is expected that solute partitioning will be lower in the surface layers of the meniscus compared to the inner core of the tissue due to the known depth-dependent differences in structure and composition. Accordingly, we investigated the partitioning of the aforementioned solutes in three distinct layers of porcine meniscus samples: the core, femoral surface layer, and tibial surface layer. Lastly, this study examined the relationship between  $K$  and water content of the tissue.

## 2. Methods

### 2.1. Sample Preparation

Porcine menisci were obtained frozen from a tissue bank (Animal Technologies Inc., Tyler, TX) and stored at  $-20^{\circ}\text{C}$  until experimentation. The middle portion of 10 unpaired medial ( $n = 5$ ) and lateral ( $n = 5$ ) menisci were cut into equal halves between the tibial and femoral surfaces. Tissue cylinders were obtained in the axial direction using a 5 mm corneal trephine with the layer of interest facing downwards to ensure a flat surface. Meniscal cylinders were inserted flat-side-first into a custom 3D printed chamber and cut into 0.5 mm thick disc samples using a razor blade, see Fig. 1. Nine samples were obtained from each meniscus (3 per layer) for a total of 90 samples. Each sample was inspected via light microscope (VWR VistaVision, Radnor, PA) to confirm isolation of surface layer from the remaining core tissue. Immediately after preparation, samples were stored at  $-4^{\circ}\text{C}$  submerged in protease inhibited phosphate-buffered saline (PBS, Sigma-Aldrich, St. Louis, MO) until testing.

### 2.2. Partition coefficient measurement

$K$  of fluorescein (10  $\mu\text{M}$ , Sigma-Aldrich, St. Louis, MO) and FITC-labeled 3-kDa and 40-kDa dextrans (3k Dex and 40k Dex, 10  $\mu\text{M}$ , Invitrogen, Waltham, MA) in meniscus were measured as previously described [23]. Briefly, samples were sequentially submerged in two 100  $\mu\text{L}$  baths for 24 h at room temperature, one containing a solute followed by one with just PBS. Following sample removal, solute concentrations in the baths were measured using a microplate reader (SpectraMax M2 Series, Molecular Devices, San Jose, CA) with a standards calibration curve, see Fig. 2.  $K$  of each solute was calculated per Equation (1):

$$K = \frac{V_2 c_2}{\Phi^w V_t (c_1 - c_2)} \quad (1)$$

where  $V_t$  is the tissue volume,  $V_2$  is the volume of the second (PBS) bath,  $\Phi^w$  is the tissue's water volume fraction, and  $c_1$  and  $c_2$  are the solute concentrations in the solute and PBS baths, respectively.

### 2.3. Water volume fraction measurement

As required for Equation (1), the  $\Phi^w$  was measured using an analytical balance with a density determination kit (Model XSR, Mettler Toledo, Columbus, OH). Samples were weighed in air ( $W_{wet}$ ), in PBS ( $W_{PBS}$ ), and after overnight lyophilization ( $W_{dry}$ ). Equation (2) was used to calculate  $\Phi^w$ ,

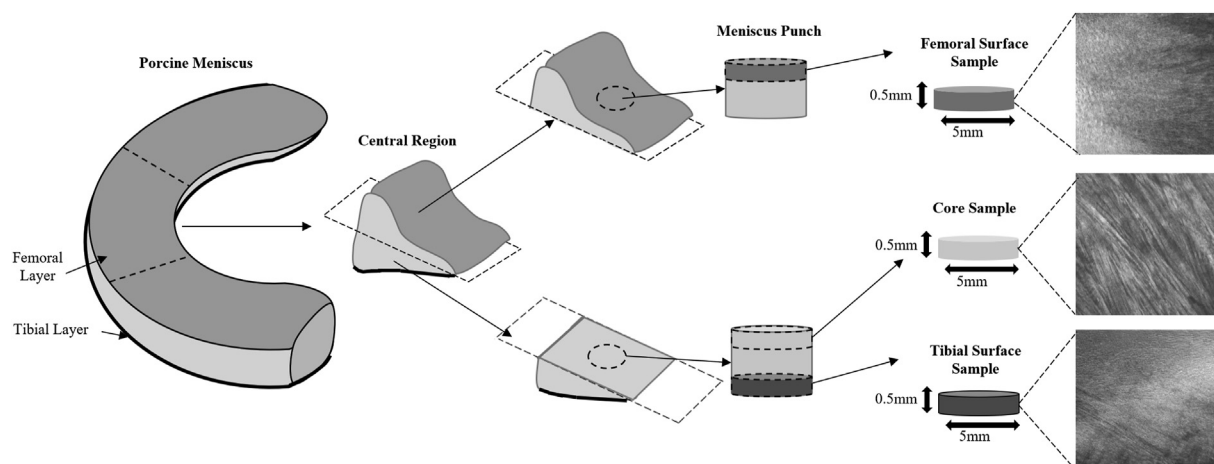
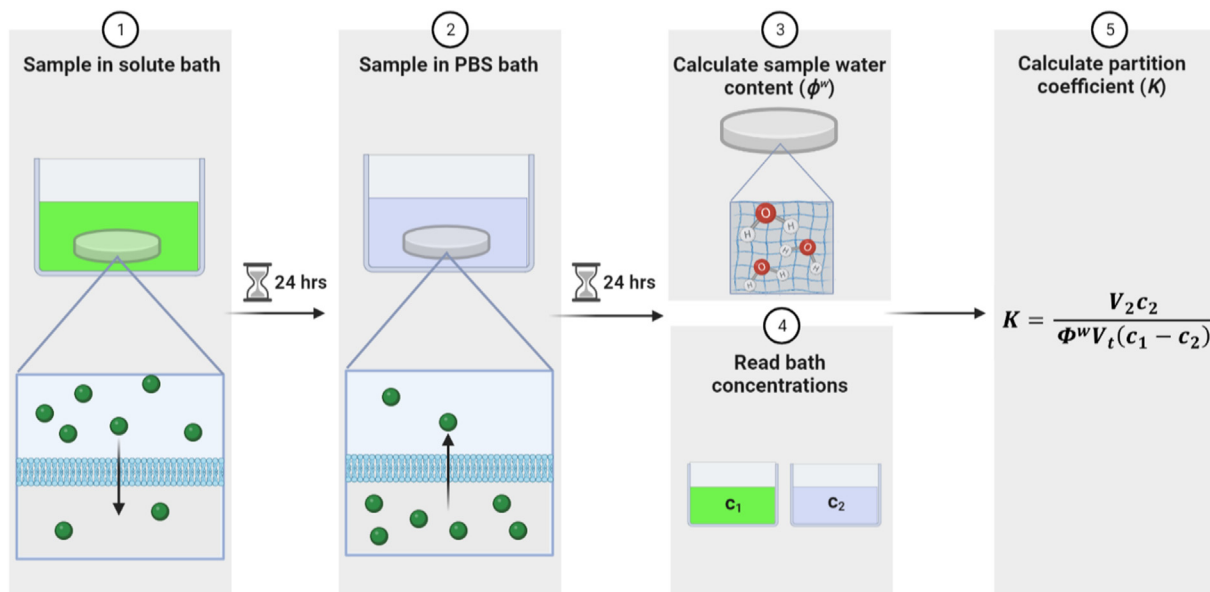


Fig. 1. Sample Preparation from porcine meniscus.



**Fig. 2.** Partition coefficient measurement summary. Meniscal samples were submerged in a solute bath followed by a PBS bath prior to lyophilization. The solute concentration in each bath was measured with a microplate reader after sample removal (Figure made with biorender: <https://biorender.com/>).

$$\phi^w = \frac{(W_{wet} - W_{dry})\rho_{PBS}}{(W_{wet} - W_{PBS})\rho_{H2O}} \quad (2)$$

where  $\rho_{H2O}$  and  $\rho_{PBS}$  are the densities of water and PBS, respectively.

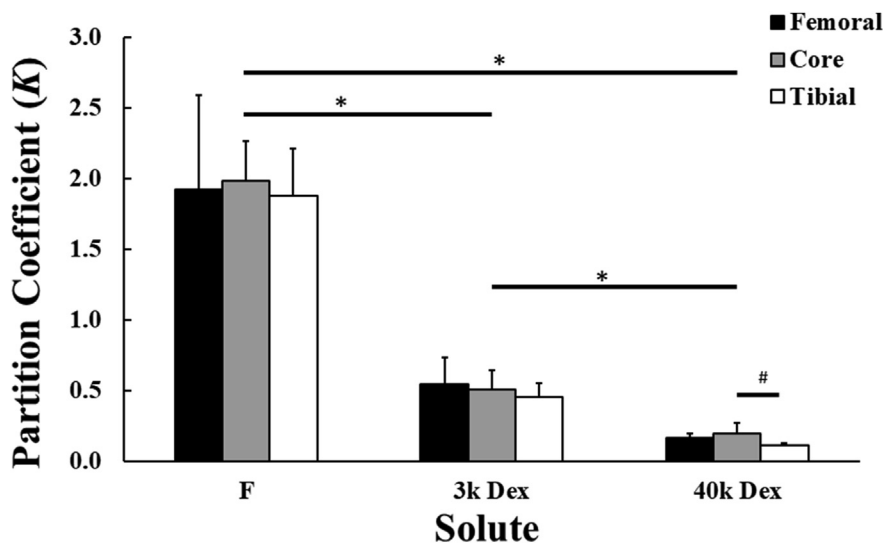
**2.4. Statistical analysis**

All statistical analyses were conducted on Minitab Statistical Software (Minitab LLC, State College, PA). A three-way ANOVA with replication and factors being meniscal layer (tibial, core, and femoral), meniscal compartment (medial and lateral), and solute (fluorescein, 3k Dex, and 40k Dex) was used to investigate possible significant interactions and main effects on  $K$ . In addition, Tukey Post Hoc tests were performed to determine significant differences between individual groups. A two-way ANOVA was adopted for investigating the effect of meniscal compartment and layer on  $\phi^w$ . Finally, linear regression analyses were performed to investigate a potential relationship between  $\phi^w$  and  $K$  for the

molecules considered. For all analyses conducted, Grubbs tests were conducted to identify any outliers. For each test conducted, a level of significance of 95% ( $\alpha = 0.05$ ) was used. Data are reported as mean  $\pm$  standard deviation.

**3. Results**

Values for  $K$  of the three solutes in each meniscal layer are summarized in Fig. 3.  $K$  ranged from a high of  $\sim 2.9$  in fluorescein to a low of  $\sim 0.1$  in 40k Dex. Results from the three-way ANOVA indicated that there were no interactions among the main effects of meniscal compartment, meniscal layer, and molecular type. Solubility measurements pooled by layer and compartment for each solute showed a decrease in  $K$  as molecular weight increased ( $p < 0.001$ ). Tukey post-hoc analysis showed differences between the fluorescein and 3k Dex ( $p < 0.001$ ), 3k Dex and 40k Dex ( $p = 0.003$ ), and fluorescein and 40k Dex ( $p < 0.001$ ). In contrast, there was no significant effect of the compartment (i.e., medial



**Fig. 3.** Partition coefficient measurements of fluorescein (F), 3 kDa dextran (3k Dex), and 40kDa dextran (40k Dex) in porcine menisci divided by layers. Medial and lateral samples were pooled together since no significant effect of compartment was observed. \* Represent statistically significant differences in  $K$  between molecules, and # represent significant differences between meniscal layers.

vs. lateral) on the measured value of  $K$  ( $p = 0.06$ ). Finally, there was no significant effect of the meniscal layers on  $K$  for fluorescein and 3k Dex. Conversely, tissue layer did significantly affect the solubility of 40k Dex with the core having the largest  $K$ .  $K$  of 40k Dex in the core layer was significantly larger than in the tibial layer ( $p = 0.032$ ) but not different from the femoral layer ( $p = 0.51$ ).

The  $\phi^w$  of all samples averaged  $0.770 \pm 0.032$ .  $\phi^w$  was significantly larger ( $p < 0.001$ ) in the medial compartment ( $0.785 \pm 0.027$ ) compared to the lateral counterpart ( $0.756 \pm 0.029$ ), see Table 1. However, differences in  $\phi^w$  were not observed across meniscal layers. Regression analyses revealed that  $K$  of 3k Dex was negatively correlated to  $\phi^w$  ( $p = 0.001$ ,  $R^2 = 32.1\%$ ). No significant correlation between  $K$  and  $\phi^w$  was found for fluorescein or 40k Dex molecules.

#### 4. Discussion

Due to its avascularity and lack of self-repair, it is important to study the partition of solutes in the meniscus. Previous studies have recorded  $K$  of the same molecules (fluorescein, 3k Dex, and 40k Dex) on articular cartilage [18,20,25]. However, to the best of our knowledge, this is the first study to characterize fluorescein and dextran partitioning in meniscus. In addition, the present study is the first to investigate solubility across different meniscal layers. Our major finding was that  $K$  was dependent on both solute size and meniscal layer for the largest solute (40k Dex). This result suggests that large drug penetration into the bulk of the meniscus will be significantly reduced by the superficial layers of the tissue.

The measurements of  $K$  reported in this study are comparable to those previously reported on articular cartilage [20,24–26]. Specifically, we found  $K$  of fluorescein was greater than 1, which is in agreement with several studies for articular cartilage [20,25,26]. In contrast, the meniscal  $K$  of glucose was found to be much lower at  $\sim 0.8$ , despite having a molecular weight half as large as fluorescein [17]. This interesting finding suggests that an attractive solute-matrix interaction may exist for fluorescein [20,25]. Furthermore, this also highlights the potential benefits of exploiting solute-matrix interactions for increasing the solubility and thus delivery of solutes into the dense extracellular matrix of the meniscus [27].

In general, larger molecules had a lower  $K$  across all tissue regions tested. The partitioning of fluorescein was the highest ( $1.93 \pm 0.47$ ), followed by 3k Dex ( $0.50 \pm 0.15$ ), which was higher than 40k Dex ( $0.16 \pm 0.07$ ). This pattern is consistent with previous solubility and diffusivity measurements in meniscus [4,28,29] and articular cartilage [24,28,30–32] in which diffusion is inversely related to solute size. Such behavior is likely due to interactions between the solute and the densely organized collagen extracellular matrix that hinder the ability of large molecules to diffuse into the tissue. This information has important implications for the development of drug delivery therapies addressing meniscal pathology, whereby drugs are delivered via intra-articular injection into the knee joint. To optimize such drug delivery into the core of the meniscal tissue, therapeutic molecules with relatively low molecular

weights are more suitable as they are less likely to be excluded due to steric hindrance of the matrix.

The effect of meniscal layer on solute partitioning varied for the molecules examined.  $K$  was not significantly affected by tissue layer for both fluorescein and 3k Dex. Conversely, 40k Dex partitioning was greatest in the core layer, and significantly higher than in the tibial layer ( $p = 0.032$ ), see Fig. 3. Our results indicate that solubility of smaller noncharged solutes ( $\leq 3k$  Da) is not reduced in the superficial layers of the meniscus; however larger solutes such as 40k Dex may face increased steric hindrance when encountering the superficial layers. This behavior is likely the result of the difference in the organization of the matrix in the different layers of the tissue. It was previously found that, in the core region, pores run parallel to the fiber bundles which have been hypothesized to facilitate diffusive transport [4,17,29]. These pores may also improve the solubility of large molecules ( $\geq 40k$  Da) in the core region by allowing their entry into the tissue. In contrast, the superficial layers of the meniscus are composed of randomly oriented collagen fibers that serve to envelop the core of the tissue and facilitate surface to surface motion [15]. This densely packed random organization may provide greater hindrance to solutes, particularly to larger molecules which may be sterically excluded from the dense matrix. From a therapeutic perspective, drug delivery treatments using large molecules targeting the core of the meniscal tissue must consider that the superficial tissue layers may serve as a barrier to entry into the bulk of the tissue.

Averaged meniscal  $\phi^w$ , pooled from all groups, was  $0.770 \pm 0.032$ , which is comparable to previous measurements on human [9,33–38] and on porcine tissues [9,39–41]. Medial meniscal samples had significantly higher  $\phi^w$  on average than lateral samples ( $p < 0.001$ ). On the other hand, no differences in  $\phi^w$  were found between the layers of each meniscal compartment. Previous water content measurements on the layers of medial bovine meniscus obtained similar results with no variations between superior and deep layers [42].  $K$  and  $\phi^w$  were negatively correlated for 3k Dex ( $p = 0.001$ ), but no correlation was found for the remaining solutes, see Fig. 4. This result suggests that 3k Dex solubility decreases with increasing tissue water content. While some previous studies have found that water content correlates with solute partition coefficient in cartilaginous tissues [17,18,23,43], others have found no correlation [44]. The specific relationship between solubility and tissue composition may be dependent on numerous factors related to the molecule of interest, including solute size, shape, and presence of solute matrix-interactions [43]; such phenomena may explain the difference in relationships between water content and partitioning for the molecules investigated here. Understanding how solute partitioning varies with tissue composition has important implications for drug delivery treatment strategies given that the composition of the meniscus changes with degeneration.

The results of this study are not only important for developing new drug delivery strategies to treat meniscus degeneration and/or lesions but could also be critical in the design of biomaterials scaffolds intended to replace meniscus tissue that is removed due to disease. When developing tissue replacements, it is essential that critical components of the native tissue be recapitulated in the scaffold in order to best mimic its function. Previous studies have shown that the outer layers of the meniscus exhibit unique mechanical properties as compared to the core portion [15,42,45–47]; our study suggests that may also be the case for transport properties, further emphasizing the importance of the particular structural arrangement of the tissue to its performance *in vivo*. In order to more fully elucidate the effect of matrix structure and composition on transport of molecules into and through the meniscus tissue, future studies are needed to investigate these properties in other regions of the tissue with distinct composition and organization (e.g., horns vs. central, inner vs. outer, etc.).

This study presented several limitations. Firstly, meniscal samples were obtained from healthy porcine tissue due to its common availability and high similarity to human tissue [48–52]. However, this limits our capacity to investigate the effects of tissue degeneration on

**Table 1**

Meniscal tissue water content as a fraction of total tissue volume. Data are divided by meniscal compartment as well as by layers within each side.

Water Volume Fraction ( $\phi^w$ )	
Combined	$0.770 \pm 0.032$
Medial	$0.785 \pm 0.027$
Femoral	$0.791 \pm 0.030$
Core	$0.784 \pm 0.026$
Tibial	$0.781 \pm 0.024$
Lateral	$0.756 \pm 0.029$
Femoral	$0.757 \pm 0.029$
Core	$0.748 \pm 0.029$
Tibial	$0.762 \pm 0.031$

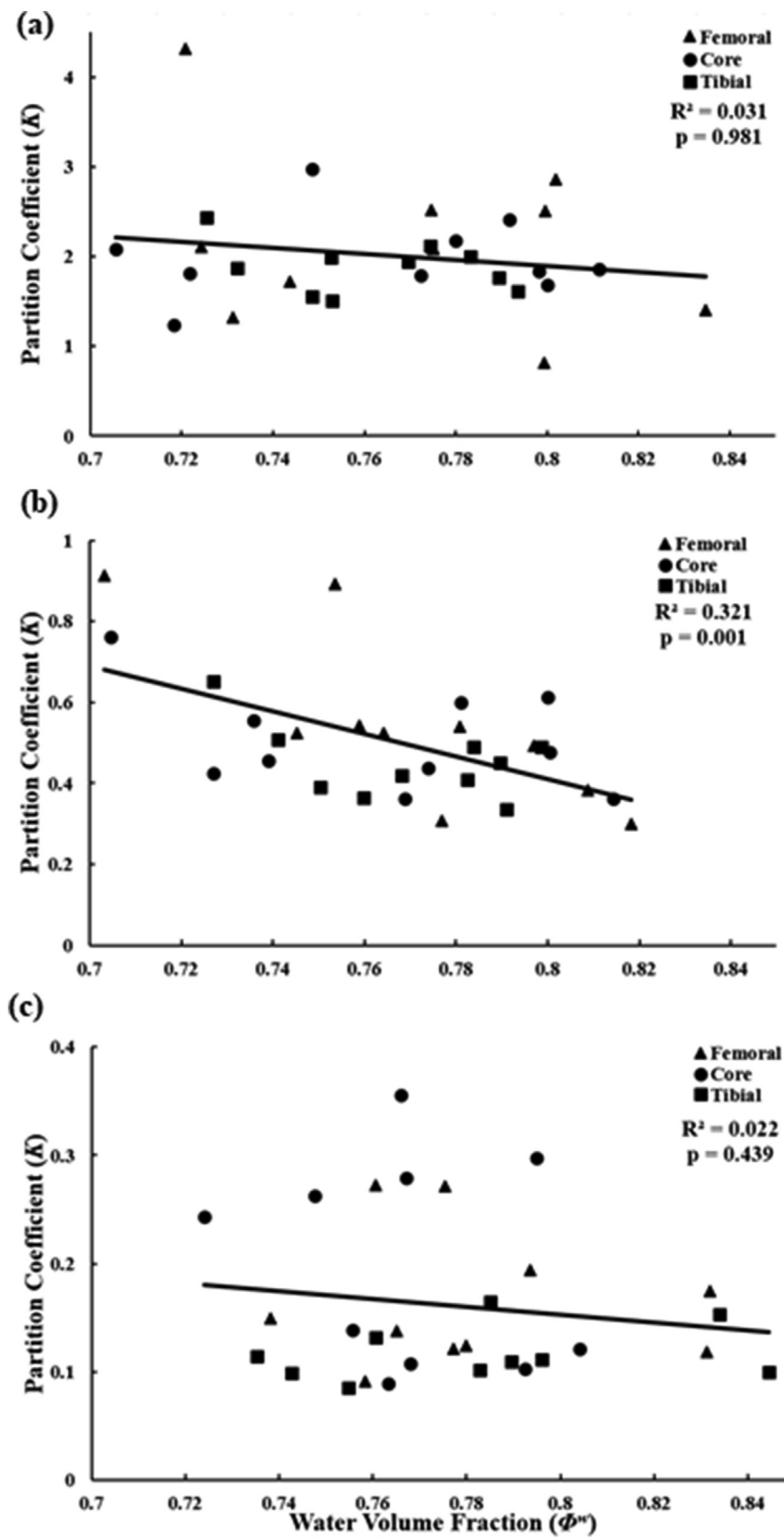


Fig. 4. Linear regression of partition coefficient against tissue water volume for each solute: (a) fluorescein (F), (b) 3k Dextran, and (c) 40k Dextran. Data were pooled from all meniscal compartments and layers.  $R^2$  values for each regression analysis is shown.



the solubility of the studied molecules. Since drug delivery therapies will likely target deteriorated joints, future studies will utilize human tissue to investigate the effect of degeneration on solute partitioning in the meniscus. Secondly, the dextran molecules (3k Dex and 40k Dex) used in the present study are linear polymeric chains [53]. While these molecules represent a large range of molecular weights, their  $K$  measurements might differ from those of globular molecules with similar molecular weights [20]. Future studies will also focus on determining the dependence of molecular shape on meniscal solute partitioning. Moreover, this study was limited to unloaded tissue samples, while the tissue undergoes a variety of loading conditions *in vivo*, including compressive, shear, and tensile loading in both static and dynamic states. Previous studies have shown that the rate of transport in articular cartilage is affected by mechanical loading, with dynamic loading augmenting the transport of large solutes [54–56], while our previous work showed that diffusion of glucose is reduced by static compression in meniscus tissue [17]. More information is needed to understand how such mechanical perturbations affect solute transport into the tissue; the present study serves as a launching point to more comprehensively investigate solute partitioning in the meniscus in future works. In addition, due to sample geometry, all samples used in this study were extracted from the central region of the menisci. Further studies to investigate the potential regional variations (i.e. central vs horns, vascular vs avascular) are required to gain a more comprehensive understanding of the diffusive properties of the meniscus given its reported homogeneity of mechanical and transport properties [36,38,48,57–61]. Finally, this study investigated solute partitioning at room temperature (~23 °C), while previous studies have shown that diffusion and solubility in cartilaginous tissues depends on temperature [20,62]. Therefore, to more fully understand transport properties in the meniscus under physiological conditions and better inform drug delivery strategies to treat the tissue, future studies should investigate the partition coefficient of solutes at body temperature (37 °C).

In conclusion, the results of this study add new knowledge on solute partitioning in meniscus and its dependence on the solute molecular weight and tissue layer. Significant decreases in  $K$  were found as the molecular weight of the molecule increased. In addition, for the largest molecules studied (40k Dex),  $K$  was lower in the superficial layers compared to the core of the meniscus. This suggests that drug delivery into the bulk of the tissue may be reduced by the presence of superficial layers of the meniscus, which could potentially act as a barrier particularly for the entrance of larger molecules. This novel information can be used to improve the design of drug delivery treatments aimed at treating knee pathologies such as deterioration of the meniscus.

#### Author contributions

Research design was conducted by AM, GS, TMB, FT, and ARJ; data acquisitions analysis was carried out by AM, GS, FT, and ARJ; data interpretation involved AM, TMB, FT, and ARJ; all authors were involved in drafting and revising the manuscript and have approved its final version for submission.

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#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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