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Investigation of the Effects of Extracellular Osmotic Pressure on Morphology and Mechanical Properties of Individual Chondrocyte

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Keywords — **Cell biomechanics, osmotic pressure, AFM, strain-rate, mechanical properties.**

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Abstract – **It has been demonstrated that most cells of the body respond to osmotic pressure in a systematic manner. The disruption of the collagen network in the early stages of osteoarthritis causes an increase in water content of cartilage which leads to a reduction of pericellular osmolality in chondrocytes distributed within the extracellular environment. It is therefore arguable that an insight into the mechanical properties of chondrocytes under varying osmotic pressure would provide a better understanding of chondrocyte mechanotransduction and potentially contribute to knowledge on cartilage degeneration. In this present study, the chondrocyte cells were exposed to solutions with different osmolality. Changes in their dimensions and mechanical properties were measured over time. Atomic Force Microscopy (AFM) was used to apply load at various strain-rates and the force-time curves were logged. The thin-layer elastic model was used to extract the elastic stiffness of chondrocytes at different strain-rates and at different solution osmolality. In addition, the porohyperelastic (PHE) model was used to investigate the strain-rate dependent responses under the loading and osmotic pressure conditions. The results revealed that the hypo-osmotic external environment increased chondrocyte dimensions and reduced Young's modulus of the cells at all strain-rates tested. In contrast, the hyper-osmotic external environment reduced dimensions and increased Young's modulus. Moreover, by using the PHE model coupled with inverse FEA simulation, we established that the hydraulic permeability of chondrocytes increased with decreasing extracellular osmolality which is consistent with previous work in the literature. This could be due to a higher intracellular fluid volume fraction with lower osmolality.**

Keywords — **Cell biomechanics, osmotic pressure, AFM, strain-rate, mechanical properties.**

1. INTRODUCTION

Living cells in the human body are subjected to various mechanical stimuli throughout their life. When cells experience mechanical forces they deform and transmit the mechanical signals to regulate biological processes. Experimental evidence has shown that cells are sensitive to their physiological environments and physical stimuli, and such biophysical reactions underlie many aspects of cell physiology such as cell deformation, adhesion, interaction, motility and signal transduction [1-3].

Several studies have demonstrated that processes, such as growth, differentiation and migration are influenced by changes in cell shape and structural integrity [3]. It has been noted that the molecular structure of the cytoskeleton as well as cellular and sub-cellular elastic responses determine the state of human health and disease [4], and that the mechanical environment significantly influences tissue health [5]. It is therefore arguable that further study of the relationship between mechanical properties and behavior of living cells under varying osmotic conditions would lead to better understanding of the mechanisms underlying the transmission, distribution and conversion of mechanical signals into biological and chemical responses.

The mechanical deformation of fluid-saturated materials such as tissues and cells under an external stimulus is governed to a significant extent by the behavior of the fluid component [6-12]. Stimulus could be in the form of mechanical, biological or chemical changes (internal or external), or a combination of all of these triggers. This study investigates the effect of intracellular fluid by studying the mechanical behavior of single living cells exposed to different osmotic pressures. At equilibrium the cell is in a state of internal thermodynamic equilibrium that is characterized by an osmotic pressure. This internal condition is physio-chemical and any changes in equilibrium value results in concomitant changes in the cell's mechanical and structural conditions. For example, cell swelling due to cell injury results in the accumulation of fluid-filled vacuoles within the cell, which may eventually rupture it [13]. We therefore alter the osmotic environment of the cell and measure the resulting responses in this study.

It is well-known that single living cells are sensitive to their physicochemical environment which influences their metabolic activity, structure and mechanical properties. Most cells of the body respond to osmotic pressure by activating some processes. The mechanisms may include the organisation of the cytoskeleton (CSK) network and provocation of several transporters in the membrane to stimulate the mobilisation of osmotically active solutes [14]. In particular, chondrocytes change their shape and volume due to the increased negative fixed-charge density when the cartilage loses water during deformation [15]. Moreover, it has been reported that the disruption of the collagen network in the early stage of osteoarthritis causes an increase in water content of the cartilage which in turn leads to a reduction of the pericellular osmolality of the chondrocytes [16]. Thus, characterisation of the mechanical properties of chondrocytes subjected to varying osmotic pressures would provide a better understanding of chondrocyte mechanotransduction and potentially contribute to knowledge on the aetiology of cartilage degeneration. The aim of this study is to investigate the effects of the extracellular osmotic pressure on the morphology and mechanical properties of single living chondrocytes.

Fluid related parameters, which are the interesting material properties of fluid-filled materials, have been widely studied in the literature [8, 17-21]. Several experiments have been conducted to experimentally determine these parameters of biological tissues [8, 20, 22]. However, it is very challenging to experimentally measure these properties for single living cells. Therefore, numerical methods and continuum mechanical models are utilized to estimate the intracellular fluid properties [9, 10, 23-25]. In a previous study, we successfully applied the porohyperelastic (PHE) model to capture the strain-rate-dependent mechanical responses of intact single chondrocytes under externally applied force [9, 10]. This model is utilized in this study to elucidate the role of intracellular fluid in cells' responses.

In the experimental component of our study, single living chondrocytes were first exposed to environments characterized by varying osmolality. The effects on cell morphology and mechanical properties were then measured. The thin-layer elastic model was used to determine the elastic properties of the chondrocytes and thus, investigate the effect of osmotic pressure on chondrocytes' mechanical properties. The PHE model coupled with inverse FEA technique is then used to probe further into the effect of extracellular osmotic pressure on the relationship between the mechanical behavior of single living chondrocytes and rate of loading.

2. MATERIALS AND MODEL

2. 1. Cell culturing and AFM sample preparation

Human primary chondrocytes were obtained from the Institute of Health and Biomedical Innovation (IHBI), QUT, Brisbane, Australia, under QUT ethics regulations. The cells used in this study were collected from all zones of the cartilage. The cells' donors were unidentifiable and they came from a long time of established cells. The chondrocytes were cultured following a culturing protocol similar to previous works [10, 26] for a week until confluent. Cells were then detached using 0.5% trypsin (Sigma-Aldrich) and seeded onto a cultured Petri dish coated with poly-D-lysine (PDL) (Sigma-Aldrich) for 1–2h. Cells were placed on the PDL surface to form a strong attachment while keeping their morphology round. Biomechanical testing was conducted at room temperature. All of the cells tested are Passage 1–2 cells.

2. 2. Sample preparation for varying osmotic pressure environments

In order to study the effect of the extracellular osmotic pressure on the elastic and viscoelastic mechanical properties of single cells, several hyper-osmotic and hypo-osmotic testing solutions were created using sodium chloride (NaCl) [27, 28]. Firstly, the iso-osmotic solution was made by adding 0.9 g of NaCl in 100 ml of deionised water. This solution has an osmolality of approximately 300 mOsm. Then, NaCl and deionised water were added to this iso-osmotic solution in order to achieve three hyper-osmotic (i.e. varying osmolality of 450, 900 and 3,000 mOsm) and two hypo-osmotic (i.e. varying osmolality of 100 and 30 mOsm) testing solutions. The 3,000 mOsm solution was included because most of the intracellular fluid is removed from the cell. As a result, we can study the effects of only the solid phase of the cells. With this method, the important role of each phase in cellular mechanical responses can be investigated. The cells were first suspended in a culture medium and seeded on a PDL-coated cultured Petri dish for one hour to allow them to attach. After that, the culture medium was changed to hyper-osmotic, hypo-osmotic, and control solutions for 30 mins to expose the cells to the osmotic environment before testing or fixation. All tests were conducted at room temperature and all the cells were at Passage 1–2.

2. 3. Mechanical loading

An Atomic Force Microscope (AFM) (Nanosurf FlexAFM, Nanosurf AG, Switzerland) was used to load the cells. A colloidal probe SHOCONG-SiO_2-A-5 (AppNano) cantilever was used in the experiment (diameter of 5 µm and spring constant of 0.3114 N/m). The spring constant was obtained through analysis of the thermal noise fluctuations prior to indentation testing. Figure 1 presents the Scanning Electron Microscope (SEM) image of the colloidal probe cantilever that was used. The experimental procedure commenced by adjusting the position of the cantilever so that the colloidal probe aligns with the central (nuclear) region of the cells nominated for indentation with using the Zeiss light microscope.

Before conducting AFM indentations, the cell's height was measured using the method proposed by Ladjal et al. [29] and described in detail in a later work by Nguyen et al. [10]. The principle involves the indentation of the cell and of the adjacent area of the substrate where the force-indentation curves are recorded. The cells' heights were then measured relative to reference contact points using the indenter, while their diameters were measured using a Leica Light Microscope M125 (Leica Microsystems).

2. 4. Cell height measurement

The chondrocyte's height was also measured using the method proposed by Ladjal et al. [29] as illustrated in Figure 2. Firstly, a light microscope was utilized to locate the AFM tip and the cells in order to bring the tip to above the central area of the cells before the indentations. Note that several positions were measured around at the central area and the maximum value of the deflection of AFM cantilever was recorded to ensure that the tip measured the (relative) highest point of the cells. The indentation was then performed on the adjacent area of the substrate to obtain the Height – Deflection curves. Next, the contact points were determined automatically using the developed MATLAB program to identify h_1 for the cell and h_2 for the substrate (see Figure 2). Finally, the cell's height was calculated as $h = h_2 - h_1$.

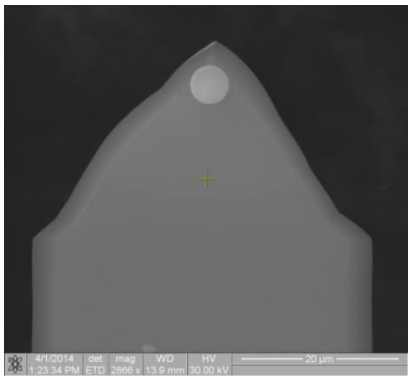

Figure 1: Scanning Electron Microscope (SEM) image of colloidal probe cantilever used in this study

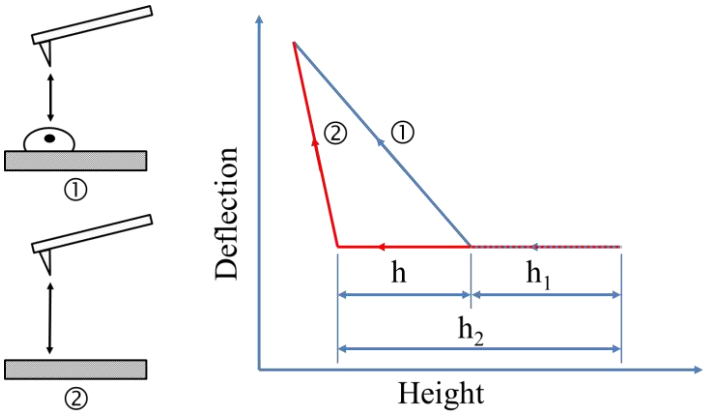

Figure 2: Cell height measurement

2. 5. Thin-layer elastic model

The elastic properties of the sample are determined from the AFM experiments using Hertzian contact mechanics which has been widely applied in AFM studies [30-32]. Hertzian theory has two major assumptions, namely linear elasticity of samples and infinite sample thickness. These two assumptions may lead to significant error [33]. Fortunately, Dimitriadis et al. [33] developed a modified Hertzian model, also known as the thin-layer model, to account for the finite thickness of a sample in AFM indentation testing. Because our samples are single living cells where the heights/thicknesses are quite thin, the so-called thin-layer model was deemed applicable. Additionally, because the single cells were attached on the substrate, the equation for the bonded

sample and substrate was also adopted. As a result, the relationship between the applied force F and indentation δ can be written as [33]:

$$F = \frac{16E_Y}{9} R^{1/2}\delta^{3/2}[1 + 1.133\chi + 1.283\chi^2 + 0.769\chi^3 + 0.0975\chi^4] \quad (1)$$

$$R = \left(\frac{1}{R_{tip}} + \frac{1}{R_{cell}}\right)^{-1} \quad (2)$$

where $\chi = \sqrt{R\delta}/h$; h, F, E_Y, R, and δ are the heights of cells, applied force, Young's modulus, relative radius (R_{tip} = 2.5 μm in this study), and indentation depth, respectively.

2. 6. PHE theory

A number of continuum mechanical models have been proposed for cell mechanics studies, one of which is the consolidation theory. The theory was originally developed for soil mechanics [34, 35] and then extended to characterize and consider the large deformation and non-linear responses of materials leading to the PHE material law [36]. With regard to cell mechanics, this theory assumes that the living cell is a continuum comprising of an incompressible mobile fluid, which is osmotically active and which flows relative to an incompressible hyperelastic porous solid skeleton. Although the solid and fluid constituents are incompressible, the whole cell is compressible due to fluid loss during deformation. The theory has been applied in many engineering fields including soil mechanics [37] and biomechanics [38-41], with the theoretical details presented by several authors [11, 38, 42-44]. The field equations for the isotropic form of this theory were presented in detail in our previous work [10]. The PHE constitutive model consists of 3 material constants: C_1 and D_1 physically represent the elastic stiffness of the solid component and the compressibility of the cell, respectively, and the hydraulic permeability k_{ij}.

The PHE model combined with the inverse FEA technique was used in this study to investigate the effect of varying extracellular osmolality on strain-rate-dependent mechanical deformation behavior of living chondrocytes. The procedure used to determine the PHE model's material parameters is similar to that presented in our previous study [10].

3. RESULTS AND DISCUSSION

3. 1 Effect of extracellular osmotic pressure on chondrocyte morphology

A total of six solutions, comprising two hypo-osmotic (i.e. 30 and 100 mOsm), one iso-osmotic (i.e. 300 mOsm) and three hyper-osmotic (i.e. 450, 900 and 3,000 mOsm) solutions were investigated. The chondrocyte diameter and height in the six different osmotic solutions were determined and shown in Figure 3, Figure 4 and Table 1. It was observed that the average height to diameter ratio of the chondrocytes was approximately one (i.e. spherical) owing to the short culture duration to which the chondrocytes were subjected. Furthermore, the volumes and apparent membrane areas of the chondrocytes were calculated from the diameter of the cells, with their values presented in Table 1 and Figure 5 for each osmolality.

As presented in Table 1, the chondrocytes underwent swelling when exposed to the hypo-osmotic solutions corresponding to a significant increase in diameter, apparent membrane area and volume. Similarly, significant decreases in diameter and volume indicated that the cells were shrinking when exposed to hyper-osmotic solutions, excluding the one with the highest osmolality (i.e. 3,000 mOsm). The possible reason is that

most of the intracellular fluid had been lost when the cells were subjected to the 900 mOsm solution. These results suggest that the osmotic environment greatly influences the morphology of the chondrocytes. The height of the chondrocytes exhibited similar changes with varying osmolality except for the case of 450 mOsm hyper-osmotic pressure where the cells did not significantly change in height relative to the iso-osmotic condition.

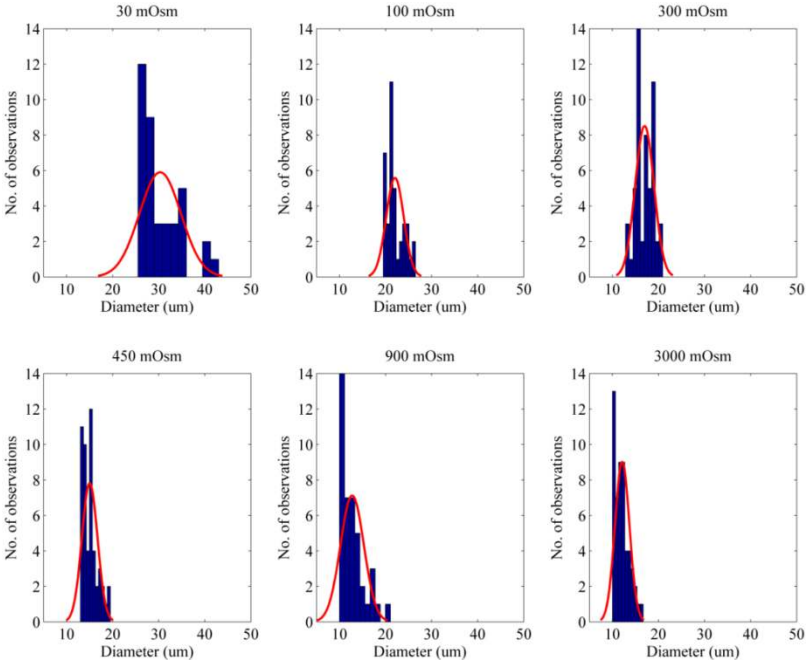

Figure 3: Diameter distributions of living chondrocytes exposed to 30, 100, 300, 450, 900 and 3,000 mOsm solutions

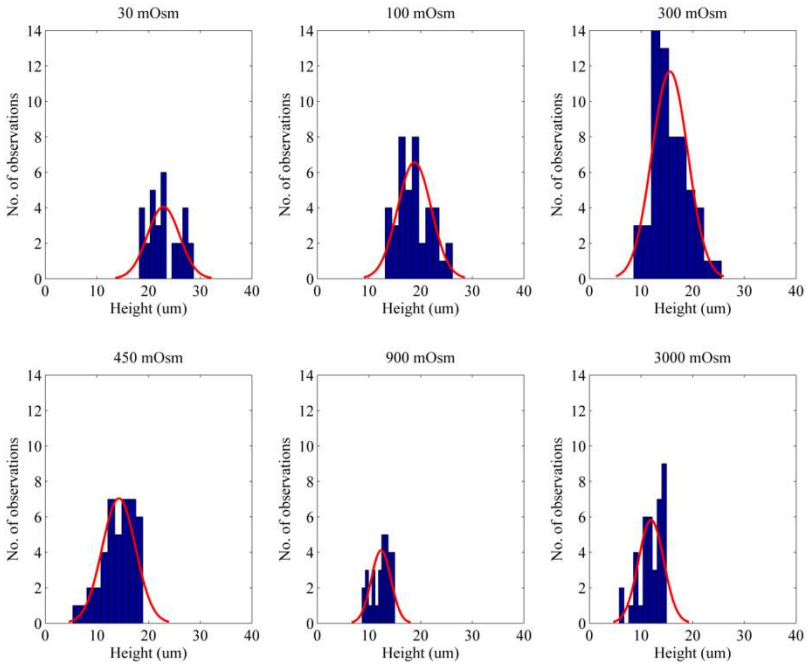

Figure 4: Height distributions of living chondrocytes exposed to 30, 100, 300, 450, 900 and 3,000 mOsm solutions

Table 1 Diameter (µm), height (µm), volume (µm^3) and apparent membrane area (µm^2) of chondrocytes exposed to 30, 100, 300, 450, 900 and 3,000 mOsm solutions

Osmolality (mOsm)	Diameter (µm)	Height (µm)	Volume (µm^3)	Membrane area (µm^2)
30	30.31 ± 4.50 (n = 38)*	22.88 ± 3.11 (n = 30)*	15,568.59 ± 7,801.56*	2,948.28 ± 927.47*
100	22.04 ± 1.90 (n = 38)*	18.8 ± 3.25 (n = 41)*	5,729.21 ± 1,552.07*	1,537.00 ± 271.17*
300	16.99 ± 2.04 (n = 54)	15.59 ± 3.47 (n = 60)	2,677.10 ± 937.39	919.77 ± 217.65
450	15.01 ± 1.69 (n = 51)*	14.26 ± 3.23 (n = 42)	1,840.70 ± 670.26*	717.06 ± 167.69*
900	12.75 ± 2.54 (n = 41)*	12.36 ± 1.90 (n = 31)*	1,223.72 ± 883.25*	530.84 ± 232.27*
3,000	12.13 ± 1.56 (n = 53)	11.95 ± 2.44 (n = 39)	982.19 ± 405.29	469.97 ± 125.02

*p < 0.05 indicated that the diameter, height and volume were significantly changed when the chondrocytes were exposed to different osmotic solutions

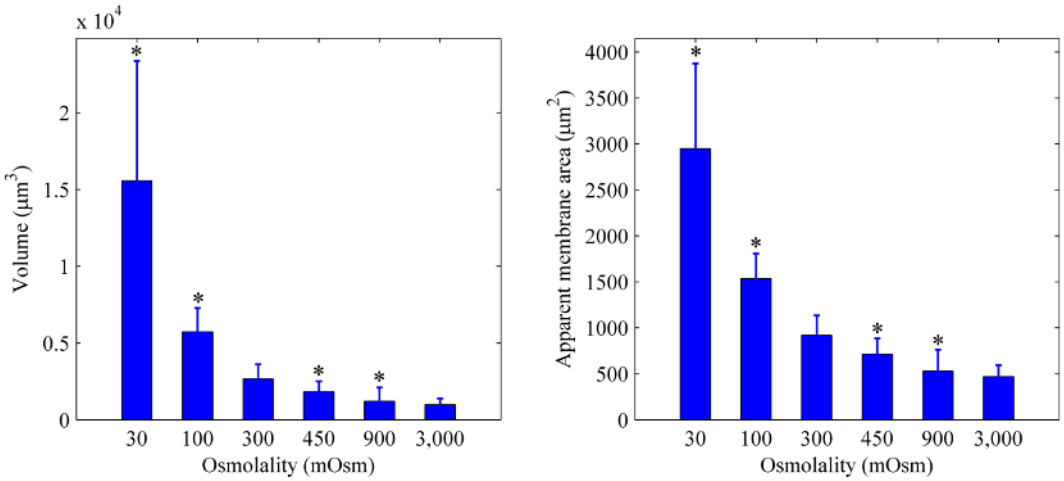

Figure 5: Chondrocyte volumes when exposed to 30, 100, 300, 450, 900 and 3,000 mOsm solutions (the data are shown as mean ± standard deviation; *p < 0.05 indicated that the volume was significantly changed)

The cellular apparent membrane area increased on average by a factor of 3.21 when the chondrocytes were subjected to the hypo-osmotic condition of 10 mOsm relative to the iso-osmotic condition in this study. This result suggests that chondrocytes have a significantly large membrane area in the control condition which is consistent with previously published work [28]. The reason suggested by previous authors was that this is because the cellular membrane consists of many folds and ruffles that can be observed in the SEM image of chondrocytes in the iso-osmotic state [28]. Thus, it is reasonable to suggest that the chondrocytes can withstand

large deformations without resulting in large stress on the cell membrane. Moreover, this finding can further support the hypothesis that the mechanical properties of living chondrocyte cells are not influenced by the membrane [28]. As a result, the cell membrane is not considered in the FEA models used in this study.

3. 2 Effect of extracellular osmotic pressure on elastic property of single chondrocytes

This study is to investigate the mechanical properties of chondrocytes at varying rates of loading and varying extracellular osmotic environments. The biomechanical properties of single living chondrocytes were quantified following exposure to six solutions of varying osmolality. Each sample was subjected to indentation on the AFM at four different strain-rates (i.e. 7.4, 0.74, 0.123 and 0.0123 s^{-1}).

In order to investigate the changes in mechanical properties, the thin-layer elastic model was used in this part of the study to estimate the Young's moduli of the living chondrocytes at each of the four strain-rates, namely, 7.4, 0.74, 0.123 and 0.0123 s^{-1}, when exposed to hyper-osmotic and hypo-osmotic solutions. The measured results are shown in Table 2 and Figure 6.

Firstly, it is interesting to note that the single living chondrocytes also exhibited strain-rate dependent mechanical deformation response when exposed to hyper-osmotic and hypo-osmotic solutions, whereby the stiffness of the cells reduced when the rate of loading decreased (see Table 2). This finding suggests that the strain-rate dependent behaviour of the cells is consistent with varying biochemical conditions and plays an important role in cellular response.

Table 2: Young's modulus (Pa) of chondrocytes exposed to 30, 100, 300, 450, 900 and 3,000 mOsm solutions at four different strain-rates (7.4, 0.74, 0.123 and 0.0123 s^{-1})

	7.4 s^{-1}	0.74 s^{-1}	0.123 s^{-1}	0.0123 s^{-1}
30 mOsm (n = 30)	367.70 ± 318.14*	301.33 ± 309.63*	225.27 ± 214.91*	156.11 ± 154.42*
100 mOsm (n = 42)	1,078.22 ± 637.49*	711.25 ± 566.56*	537.63 ± 379.00*	392.76 ± 236.41*
300 mOsm (n = 43)	1,641.55 ± 889.56	1,215.52 ± 822.26	944.13 ± 704.17	628.89 ± 493.35
450 mOsm (n = 37)	1,710.68 ± 1,429.43	1,163.40 ± 988.46	822.49 ± 738.93	643.46 ± 564.85
900 mOsm (n = 30)	1,729.81 ± 1,121.49	1,288.22 ± 912.17	985.38 ± 851.06	672.10 ± 604.02
3,000 mOsm (n = 30)	2,804.76 ± 2,648.00*	2,275.53 ± 2,395.30*	1,901.17 ± 2,191.66*	1,805.65 ± 2,041.68*

* $p < 0.05$ indicated that the Young's modulus of the chondrocytes significantly changed when the cell was exposed to varying osmotic pressure conditions

As presented in Figure 6, living chondrocytes experienced similar Young's modulus changes and behaviour at each strain-rate when exposed to varying osmotic environments. When the cells were subjected to hypo-osmotic solutions (i.e. 30 and 100 mOsm), the stiffness of the chondrocytes reduced significantly compared to the chondrocytes in the control condition (i.e. 300 mOsm) ($p < 0.05$, Table 2) at all strain-rates tested. In addition, the stiffness of the single chondrocytes significantly reduced when exposed to the hypo-osmotic solution of 30 mOsm compared to the stiffness when exposed to another hypo-osmotic solution (i.e. 100 mOsm).

On the other hand, the chondrocytes exhibited more complicated mechanical properties when exposed to the hyper-osmotic solutions. The cells did not show significant difference in elastic modulus when the osmolality of the environment changed from 300 to 900 mOsm (see Figure 6). These results are consistent with those reported in previous research [28]. Guilak et al. working with cells in an osmotic solution of approximately 466 mOsm concluded that the hypo-osmotic pressure significantly reduced the elastic modulus of single living chondrocytes whereas the hyper-osmotic pressure did not significantly affect the Young's modulus of the cells compared to the iso-osmotic condition. In this study, the hyper-osmotic pressure was increased to even higher osmolality (around 900 and 3,000 mOsm). It is interesting to note that the hyper-osmotic pressure did not have a significant effect on the chondrocytes at up to 900 mOsm. The living chondrocytes' stiffness, however, was significantly increased when the cells were subjected to the highest solution osmolality (3,000 mOsm) ($p < 0.05$, Table 2). Based on the results reported in this section, it can be concluded that all the extracellular osmotic pressures tested significantly altered the elastic stiffness of single living chondrocytes. These findings indicate that physico-chemical environment affects the cell's morphology and mechanical responses. It can also be revealed that the changes occurring in the microenvironment of chondrocytes due to osteoarthritis or deformation of the extracellular matrix may directly alter the mechanical responses of the cell [5, 16, 28].

3. 3 PHE analysis of strain-rate dependent mechanical behaviour of single living chondrocytes exposed to varying extracellular osmotic pressure conditions

One of the most interesting and important parameters in cell biomechanics is hydraulic permeability, which is very difficult to measure experimentally. Numerical simulations are therefore potential methods to estimate cell's permeability. Using combined experiments and numerical modelling offers significant benefits in cell biomechanics studies.

To extend our knowledge on the effects of the osmotic environment on chondrocytes, our current investigation was extended to include the effect of extracellular osmotic pressure on PHE material parameters (especially the hydraulic permeability) of single chondrocytes. Moeendarbary et al. [23] reported that the poroelastic diffusion constant of the cells reduced with decreases in the fluid volume fraction. It is hypothesised that the hydraulic permeability of single living chondrocytes also changes when exposed to varying osmotic pressure conditions. Therefore, the PHE model coupled with the inverse FEA technique has been applied in this study to investigate the dependence of the hydraulic permeability of chondrocytes on extracellular osmotic pressure. One of the advantages of the approach is that the permeability of the cells can be estimated based on AFM indentation testing at various strain-rates. This study is one of the first to calculate cell permeability for a wide range of strain-rates.

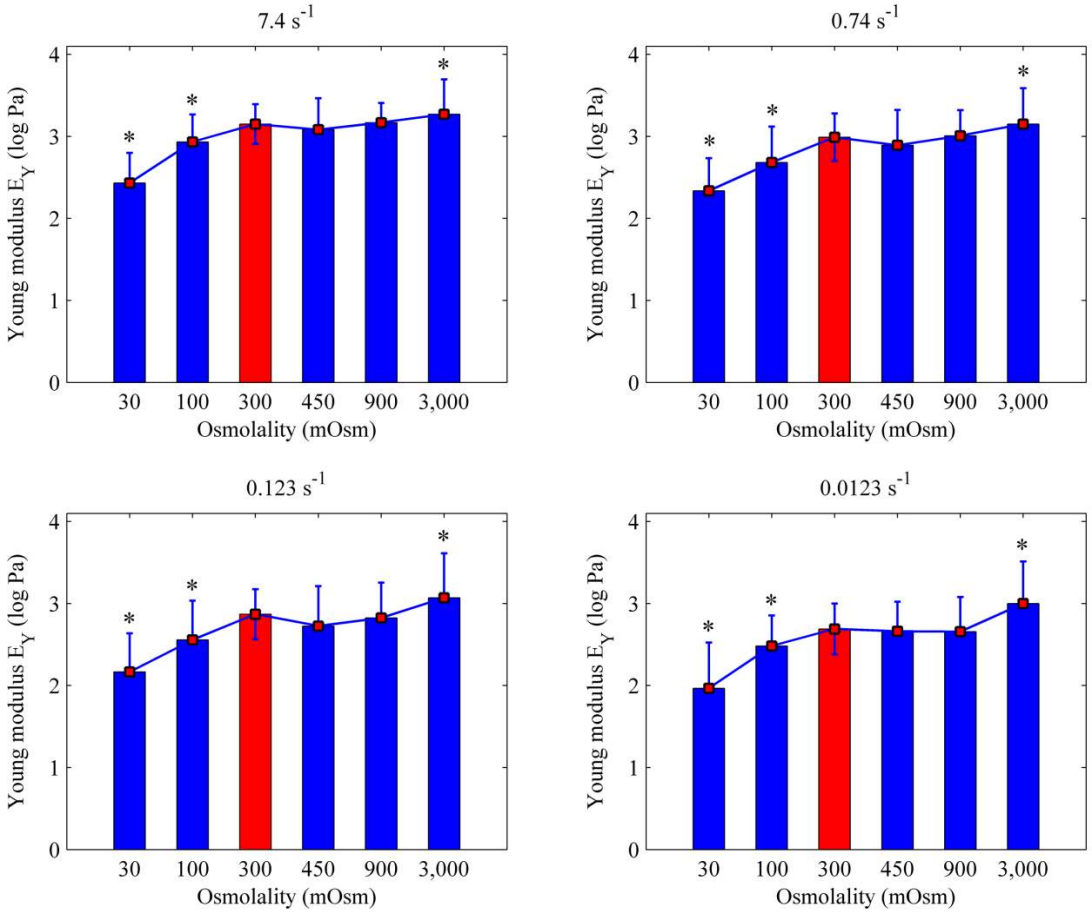

Figure 6: Young's moduli of chondrocytes at four different strain-rates (7.4, 0.74, 0.123 and 0.0123 s^{-1}) when exposed to varying osmotic environments (30, 100, 300, 450, 900 and 3,000 mOsm)

As noted above, the chondrocytes' properties significantly changed when the cells were exposed to all the hypo-osmotic solutions tested compared to their properties in the iso-osmotic condition. However, only one hyper-osmotic solution (3,000 mOsm) affected the cells' properties. Thus for simplification purposes only four solutions are investigated without lack of generality, comprising two hypo-osmotic solutions (i.e. 30 and 100 mOsm), one iso-osmotic solution (i.e. 300 mOsm) and one hyper-osmotic solution (i.e. 3,000 mOsm).

The technique presented in our previous study [10] was applied to estimate the PHE material parameters of the living chondrocytes exposed to 30, 100, 300 and 3,000 mOsm conditions. The AFM indentation biomechanical testing data at four different strain-rates was used in this investigation. The diameters of the chondrocytes presented in Section 3. 1 and in Table 1 were used to develop the FEA models of the cells shown in Figure 7. The chondrocytes were assumed to be spherical at four different osmotic solutions because the differences between diameters and heights of the cells are negligibly small.

Table 3 and Figure 8 present the PHE material parameters of the chondrocytes when exposed to the four different osmotic solutions. It was observed that the C_1 values increased with increasing solution osmolality. This finding suggested that the instantaneous modulus of the living chondrocytes was altered when the cell was exposed to varying osmotic pressure conditions, which was similar to the results at the highest strain-rate (i.e. 7.4 s^{-1}) reported in previous section. Moreover, it is interesting to note that the hydraulic permeability of the chondrocytes was significantly increased when the cells were exposed to the hypo-osmotic solutions (i.e. 30 and

100 mOsm) compared to the iso-osmotic condition. These findings are consistent with those reported in a previous investigation [23] which reported that the diffusion constant increased when the intracellular fluid volume fraction increased. In contrast, the chondrocytes did not experience a significant change in hydraulic permeability when exposed to the hyper-osmotic solution (i.e. 3,000 mOsm) ($p = 0.786$). This can be explained by the fact that most of the intracellular fluid had been lost when the cells were subjected to this hyper-osmotic solution. Thus, the mechanical properties of the cells were mainly governed by the solid phase of the cells. As presented in the previous section (Section 3. 2) and above in this section, it can be concluded that a volume increase of chondrocytes increases the hydraulic permeability but reduces Young's modulus and that a volume decrease of chondrocytes leads to an increase of the Young's modulus and unchanged hydraulic permeability.

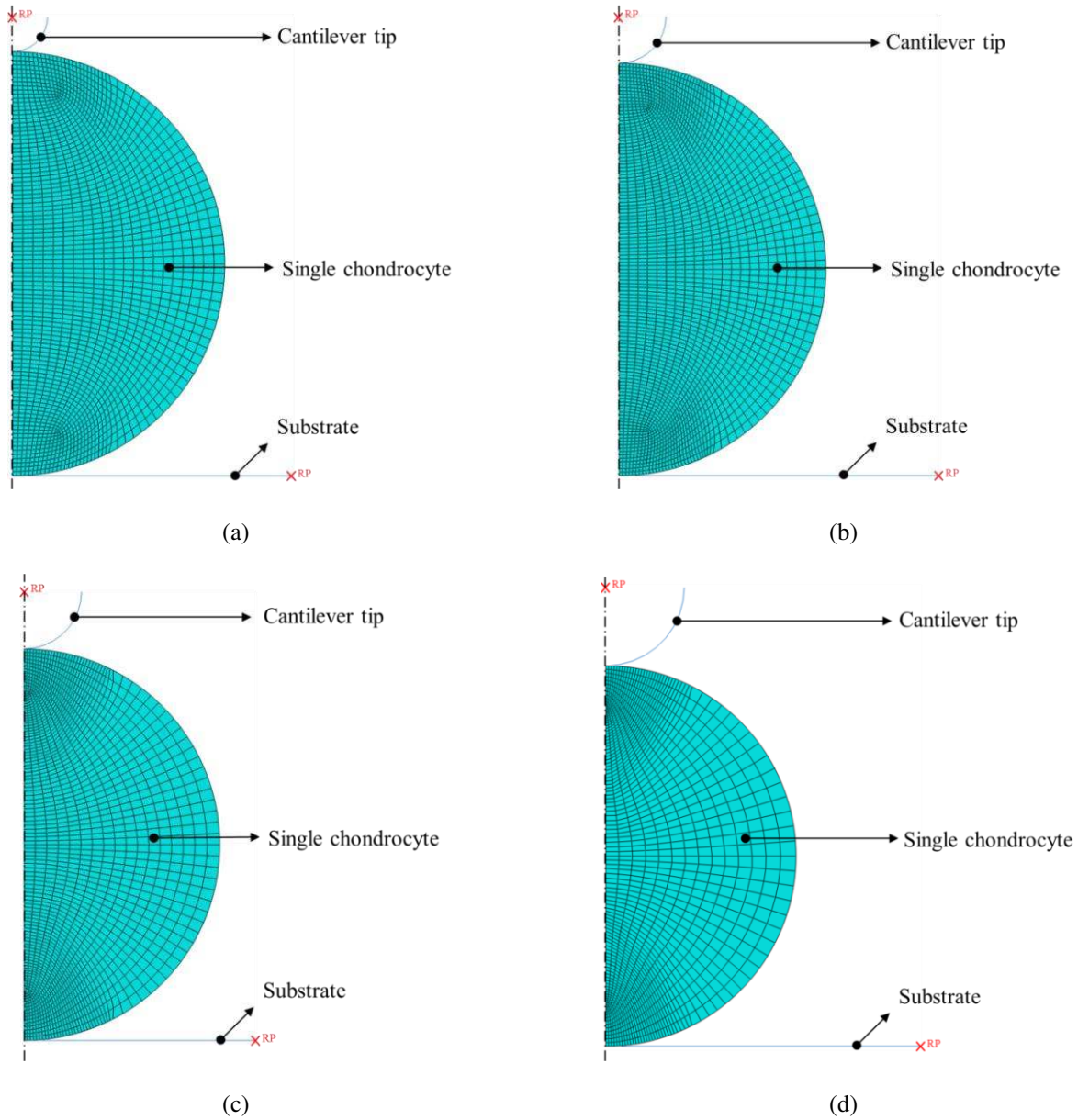

Figure 7: FEA models of single chondrocytes exposed to (a) 30, (b) 100, (c) 300, and (d) 3,000 mOsm solutions

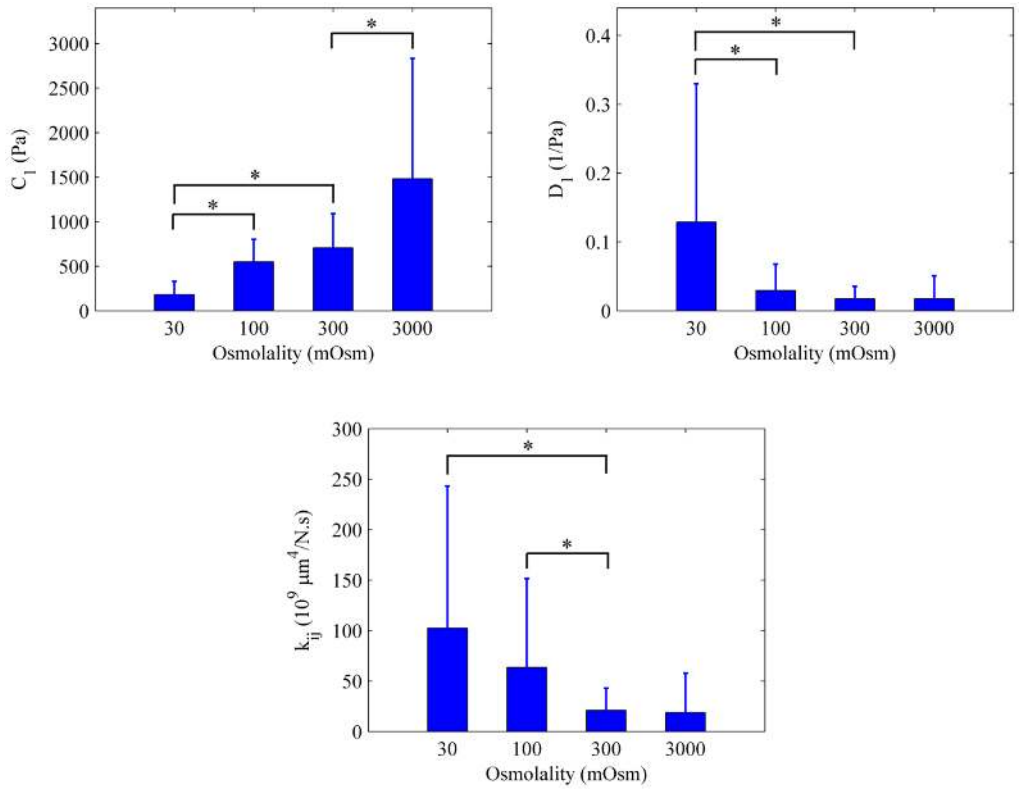

Figure 8: PHE material parameters of single living chondrocytes subjected to varying extracellular osmolality – including 30 and 100 mOsm (hypo-osmotic condition), 300 mOsm (isosmotic condition) and 3,000 mOsm (hyper-osmotic condition) (the data are shown as mean ± standard deviation; *p < 0.05 indicated the significant difference in the PHE parameters at the osmotic pressure conditions compared to other conditions)

Figure 9 presents the AFM experimental data at four strain-rates and the PHE simulation results of typical chondrocytes when exposed to four different osmotic solutions. It can be seen that the PHE model was able to effectively capture the consolidation-dependent behaviour of the chondrocytes when exposed to varying extracellular osmotic pressure conditions. Thus, it can be concluded again that the PHE constitutive model is an adequate constitutive model for simulating the strain-rate dependent properties and other behaviour of single cells.

Table 3: PHE material parameters of living chondrocytes when exposed to four varying extracellular osmotic pressure conditions

Osmolality	C_1 (Pa)	D_1 (10^{-3} 1/Pa)	Initial permeability k_0 (10^9 µm^4/N.s)	Initial void ratio e_0
30 mOsm	181.56 ± 148.21	129.00 ± 201.00	102.54 ± 140.53*	4
100 mOsm	584.67 ± 253.87	29.40 ± 38.40	63.53 ± 87.96*	4
300 mOsm	706.60 ± 384.70	17.50 ± 17.80	20.90 ± 22.00	4
3,000 mOsm	1,483.80 ± 1,348.10	17.60 ± 33.20	18.76 ± 39.07	4

* p < 0.05 indicated that the hydraulic permeability of the living chondrocytes was significantly increased when exposed to the hypo-osmotic solutions compared to the iso-osmotic condition.

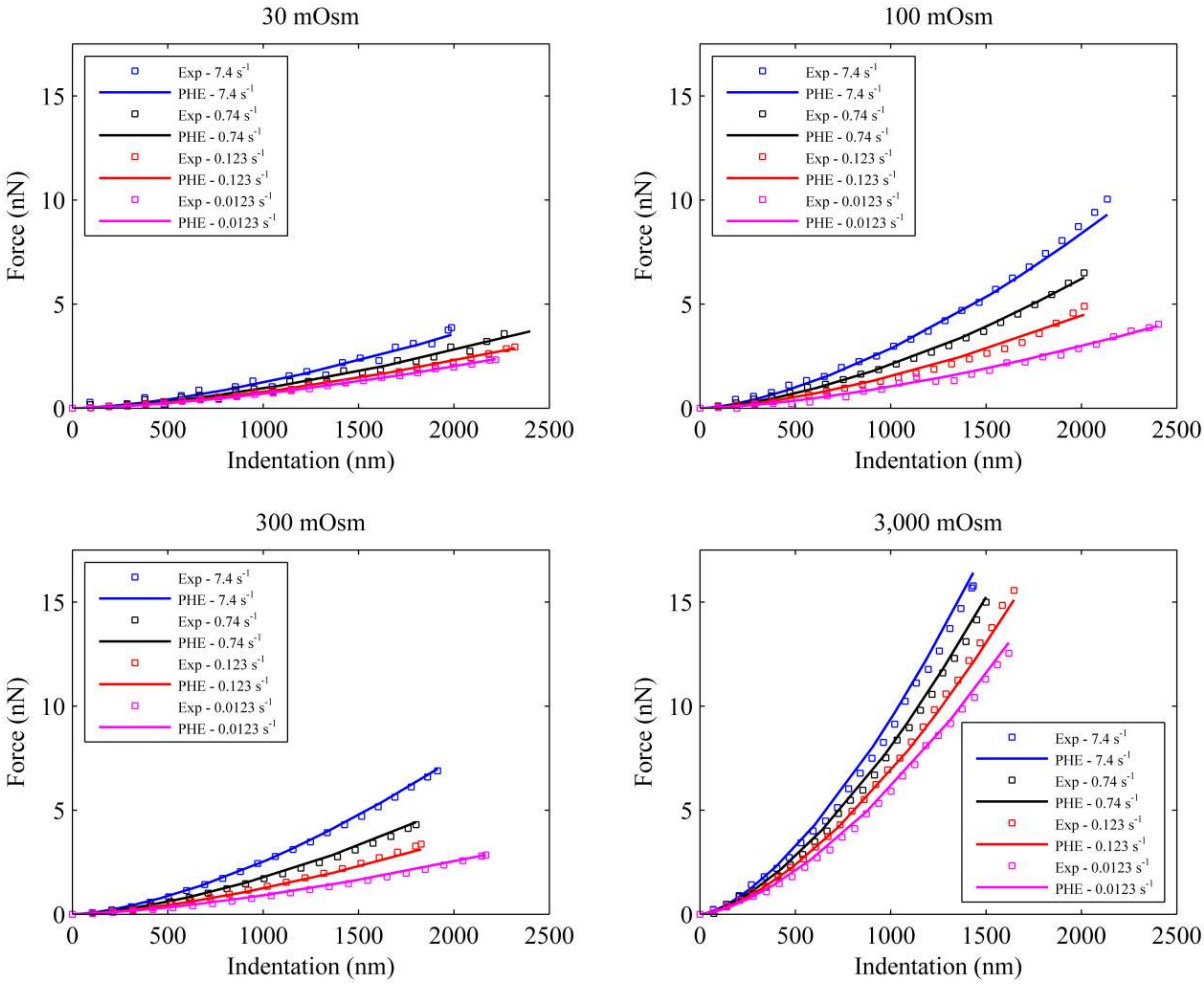

Figure 9: Experimental and PHE force–indentation curves at four different strain-rates of typical single living chondrocytes subjected to four varying osmotic pressure conditions (i.e. 30, 100, 300 and 3,000 mOsm)

4. CONCLUSIONS

The mechanical responses of chondrocytes exposed to varying extracellular osmotic pressure conditions have been studied. The thin-layer elastic was applied to determine the elastic properties of single living chondrocytes for each of the osmotic solutions tested. The PHE model was also used to study the strain-rate-dependent mechanical response of the cells. Several conclusions have been drawn as follows:

- The hypo-osmotic external environment increased the diameter, height and volume of the living chondrocytes, whereas the hyper-osmotic condition reduced the diameter, height and volume of the living chondrocytes. The AFM indentation experimental results showed that hypo-osmotic extracellular osmotic pressure conditions caused a significant reduction in the chondrocyte stiffness. However, the Young's modulus of the chondrocytes exhibited a more complicated trend when the cells were exposed to hyper-osmotic solutions. The chondrocytes did not show significant change in Young's modulus when exposed up to 900 mOsm. However, when the osmolality was increased to 3,000 mOsm, the chondrocytes' elastic moduli significantly increased. To the best of our knowledge, this is an interesting result that has not been published to date.

- These findings suggest that the extracellular osmotic pressure condition which is either hypo-osmotic or hyper-osmotic might significantly alter not only the morphology but also the mechanical properties of single living chondrocytes. This indicates the important role of intracellular fluid in the cells.

- The effect of extracellular osmotic pressure on the PHE material parameters of chondrocytes, especially the hydraulic permeability, was also investigated in this study. It was found that the decreasing extracellular osmolality reduced the elastic stiffness and increased the hydraulic permeability, whereas the increasing extracellular osmolality increased the elastic stiffness and kept the hydraulic permeability of chondrocytes unchanged. This might have been due to the changes in the intracellular fluid volume fraction when the cells were exposed to different solution osmolalities.

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