 MCB, vol.6, no.2, pp.113-119, 2009

Aging-Related Differences in Chondrocyte Viscoelastic Properties

Nikolai Steklov*, Ajay Srivastava*, K.L.P. Sung†, Peter C. Chen†
Martin K. Lotz* and Darryl D. D'Lima‡

Abstract: The biomechanical properties of articular cartilage change profoundly with aging. These changes have been linked with increased potential for cartilage degeneration and osteoarthritis. However, less is known about the change in biomechanical properties of chondrocytes with increasing age. Cell stiffness can affect mechanotransduction pathways and may alter cell function. We measured aging-related changes in the biomechanical properties of chondrocytes. Human chondrocytes were isolated from knee articular cartilage within 48 hours after death or from osteochondral specimens obtained from knee arthroplasty. Cells were divided into two age groups: between 18 and 35 years (18 – 35); and greater than 55 years (55+) of age. The 55+ group was further subdivided based on visual grade of osteoarthritis: normal (N) or osteoarthritic (OA). The viscoelastic properties of the cell were measured using the previously described micropipette cell aspiration technique. The equilibrium modulus, instantaneous modulus, and apparent viscosity were significantly higher in the 55+ year age group than in the 18 – 35 age group. On the other hand, no differences were found in the equilibrium modulus, instantaneous modulus, or apparent viscosity between the N and OA groups. The increase in cell stiffness can be attributed to altered mechanical properties of the cell membrane, the cytoplasm, or the cytoskeleton. Increased stiffness has been reported in osteoarthritic chondrocytes, which in turn has been attributed to the actin cytoskeleton. A similar mechanism may be responsible for our finding of increased stiffness in aging chondrocytes. With advancing age, changes in the biomechanical properties of the cell could alter molecular and biochemical responses.

* Shiley Center for Orthopaedic Research and Education at Scripps Clinic, La Jolla, CA
† University of California, San Diego, La Jolla, CA
‡ Corresponding Author. Shiley Center for Orthopaedic Research and Education at Scripps Clinic, 11025 North Torrey Pines Road, Suite 140, La Jolla, CA 92037. Tel: 858-332-0166; Fax: 858-332-0140; Email: ddlima@scripps.edu

1 Introduction

Aging is a major risk factor for primary osteoarthritis (OA), which is one of the most common forms of OA. Aging also accelerates secondary forms of OA such as posttraumatic OA (1). Articular cartilage changes profoundly with aging. Cell density and cartilage thickness is reduced while stiffness increases. There is a general reduction in the proliferative responses of articular chondrocytes with aging (2-6). Induction of extracellular matrix synthesis by growth factors is also impaired (7-9). Hence, changes at the cellular level may be responsible for aging-related cartilage degeneration at the tissue and joint levels. However, little is known of the biomechanical changes that occur with chondrocyte aging at the cellular level.

The chondrocyte cytoskeleton is a three-dimensional network composed of three major elements: actin microfilaments, vimentin intermediate filaments, and tubulin microtubules. While all three cytoskeletal proteins have been implicated in the biomechanical properties of the cell, actin is the most widely studied and appears to contribute the most to cell stiffness (10-13). Actin filaments play a role in a variety of cell functions, including, cell migration, cell adhesion, shape alteration, cell signaling, organelle movements, endocytosis, secretion, contractile ring formation, myocyte contraction, extracellular matrix assembly, and regulation

of chondrocyte hypertrophy (14-19). Therefore changes in actin could result in biomechanical changes in chondrocytes and, more importantly, be reflected in altered cell function.

Micropipette aspiration of cells in suspension is an well characterized method of measuring elastic and viscous properties of cells (20). On micropipette aspiration osteoarthritic chondrocytes are stiffer than normal (21). This increased stiffness has in part been attributed to actin microfilaments (10). However, whether the stiffness of chondrocytes changes with aging is not known. This study was therefore designed to measure biomechanical properties of chondrocytes obtained from donors from different age groups to test the hypothesis that aging significantly affects the viscoelastic properties of chondrocytes.

2 Materials and Methods

Human articular chondrocytes were isolated from visually normal femoral and tibial articular cartilage of healthy cadaver donor knees within 48 hours after death (obtained from tissue banks) or from excised osteochondral tissue from patients undergoing total knee arthroplasty. Chondrocytes were resuspended at a density of 5×10^5 cells per mL in Dulbecco's Modified Eagle Medium. Cells were divided into groups based on the age of the donor: between 18 and 35 years (18 – 35); and greater than 55 years (55+) of age. The 55+ group was further subdivided based on visual grade of osteoarthritis. Cells obtained from cartilage that had an intact surface zone (Grade I) or had mild surface fibrillations (Grade II) were classified as Normal (N). Cells obtained from cartilage with severe surface fibrillation, or partial thickness loss of cartilage (Grade III), or adjacent to a full-thickness lesion (Grade IV) were classified as osteoarthritic (OA). All of the cartilage samples from the 18 – 35 age group were classified as Normal (Grade I or II). A total of 33 cells were obtained from 5 donors in the 18 – 35 age group, a total of 46 cells were obtained from 9 donors in the Low Grade 55+ group, and a total of 26 cells were obtained from 5 donors in the High Grade 55+ group. In a subset of 3 donors from the High Grade 55+ group, cells obtained from Grade III areas and cells from Grade I or II areas were separately tested in an attempt to control for age and donor to donor variability.

Micropipette aspiration and manipulation were performed using a similar technique to that described previously (21, 22). Briefly, micropipettes were made by drawing thin-walled glass capillary tubes (Sutter Instruments, P 87) and fracturing them on a microforge (F9, Narishige Co. Ltd.) to create an inner diameter of approximately 5.5 microns. The selected chondrocyte from the cell suspension was initially stabilized against the micropipette by applying a tare pressure of 0.09k Pa for 60 seconds followed by a step pressure of 0.7 kPa for 180 seconds. Video images of cell and micropipette tip were captured at 30 frames per second at 100× magnification (Fig 1). Cell aspiration length was measured from captured frames using image analysis (Matlab, Mathworks, Natick, MA) after correcting for lens artifact (due to the difference in the refractive index between the micropipette glass and the surrounding buffer).

The viscoelastic behavior of the cell was modeled using a three-parameter viscoelastic model (standard linear solid, Fig 2). The experimental data (aspiration length over time) were fit to an analytic solution of micropipette aspiration given by equations 1 and 2 (12). The viscoelastic constants (k1, k2, μ) and relaxation time constant (τ) were obtained from the curve-fit solution. The constant k1 represents the equilibrium modulus, k1 + k2 represent the instantaneous modulus, and μ is the apparent viscosity.

$$L = \frac{2a\Delta P}{\pi k1}\left(1 - \frac{k2}{k1+k2}e^{-t/\tau}\right) \tag{1}$$

$$\tau = \frac{\mu(k1+k2)}{k1k2} \tag{2}$$

2.1 Statistical analysis

Student's t test was used to determine statistically significant differences in dependent variables (equilibrium and instantaneous modulus, and apparent viscosity) between the 18 – 35 group and the 55+ group, and between the Normal and

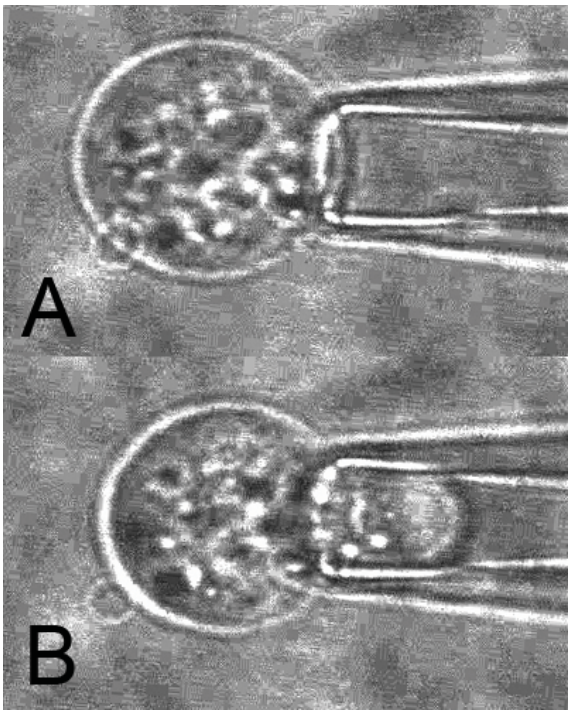


Figure 1: A: Video image of micropipette applying tare pressure (0.09k Pa) to a chondrocyte in suspension. B: Image after 120 seconds of constant pressure of 0.7 Pa showing the length of the cell aspirated into the micropipette.

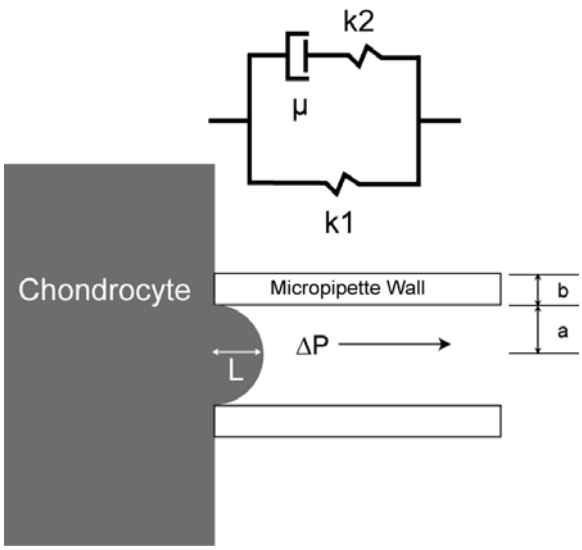


Figure 2: The viscoelastic properties of a chondrocyte were represented using a standard linear solid model (k1 and k2 represent linear springs while μ is the apparent viscosity). A theoretical formulation of the micropipette aspiration (12) was used to calculate k1, k2 and μ from the experimental data (see text for details). L is the aspirated length of the cell, ΔP is the hydrostatic pressure differential, a is the inner radius of the micropipette, and b is the thickness of the micropipette wall.

OA subgroups of the 55+ group. A p value of 0.05 was used as a threshold for significance.

3 Results

There was a distinct difference in cell aspiration length over time between the 18 – 35 and 55+ groups (Fig 3). The initial slope of the aspiration curve for 18 – 35 cells was steeper and aspiration length at equilibrium was greater than that for 55+ cells. This indicates that the 55+ cells were stiffer and more viscous than the 18 – 35 cells.

Table 1

Groups:	**18 – 35**	**55+ Normal**	**55+ OA**
Cell Size (μm)	13.8 $\pm$ 1.5	14.73 $\pm$ 2.3	15.45 $\pm$ 1.02

Statistical Significance:
18 – 35 vs. 55+ Normal, p = 0.03
18 – 35 vs. 55+ OA, p $<$ 0.001
55+ Normal vs. 55+ OA, p = 0.06

These differences on observed aspirated length were confirmed by comparison of the parameters after fitting the data. The equilibrium modulus was significantly higher in the 55+ year age group than in the 18 – 35 age group (Fig 4, p=0.001). Since the Young's modulus (E) is 1.5xk1 (12), E was also significantly higher in the 18 – 35 group. No statistically significant differences were found between Low Grade and High Grade OA subgroups of the $>$55 year age group. Similarly the instantaneous modulus (k1 + k2) was also significantly higher in the $>$55 year age group, but did not change with OA grade (Fig 4). The apparent viscosity (μ) also increased with age but not with OA grade (Fig 4). Cell size was significantly different between 18 – 35 and 55+ Normal and between 18 – 35 and 55+ OA groups (Table 1). No statistically significant differences were noted in

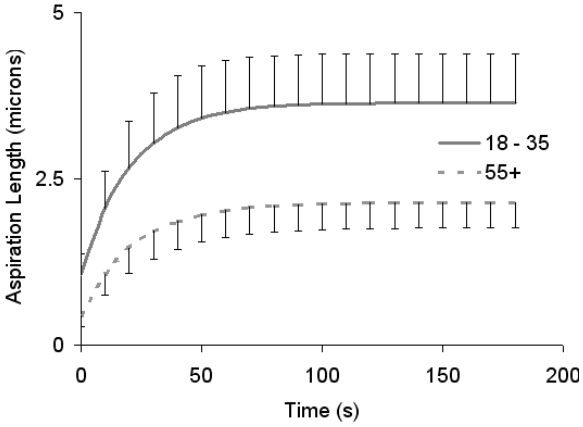


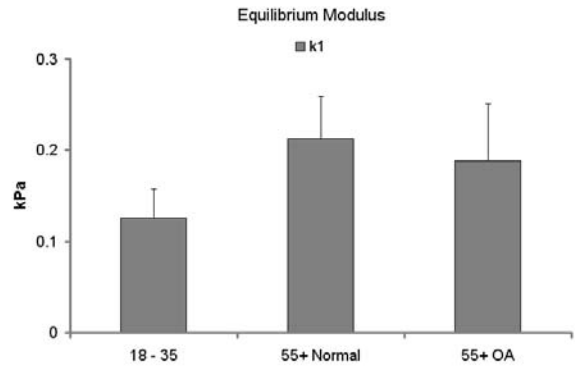
Figure 3: Comparison of aspiration lengths of 18 – 35 and 55+ groups (mean and standard error bars). Chondrocytes from the 18-35 group were aspirated further into the micropipette than chondrocytes from the 55+ group. The rate of aspiration for chondrocytes from the 18 – 35 group was also greater.

cells obtained from Normal and OA tissue from the same donors (Fig 4E).

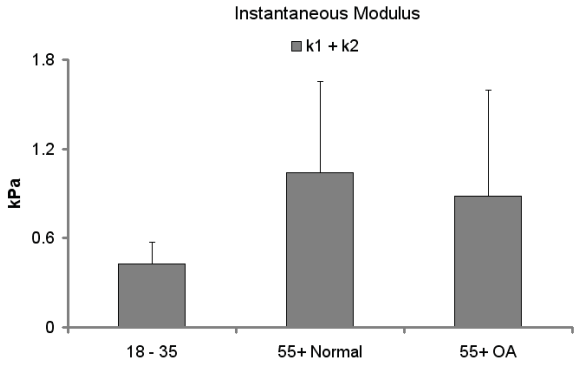
4 Discussion

Aging is associated with significant changes in articular cartilage at the cell, matrix, and tissue levels. These aging-related changes increase the susceptibility for degeneration and osteoarthritis. Aging may change the viscoelastic properties of cells, which can in turn influence mechanotransduction pathways and cellular response. To our knowledge, this is the first reported study of differences in the viscoelastic behavior of human articular chondrocytes with aging.

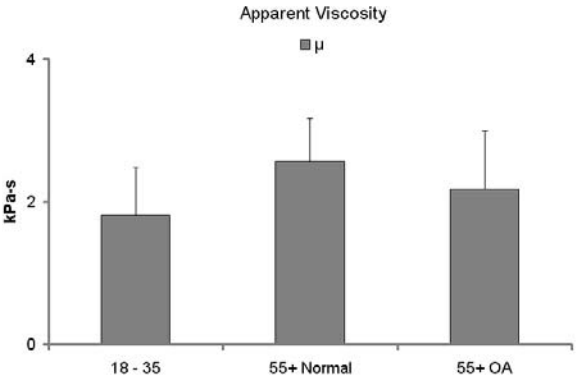
We used the micropipette aspiration technique to measure the biomechanical properties of isolated chondrocytes from donors of different age groups. Several models exist for describing the biomechanical behavior of cells undergoing micropipette aspiration. The fluid-based models treat the cell primarily as a fluid with or without cortical tension at the cell membrane (23-25). The solid models assume that the cell is an elastic or viscoelastic homogenous solid (12,



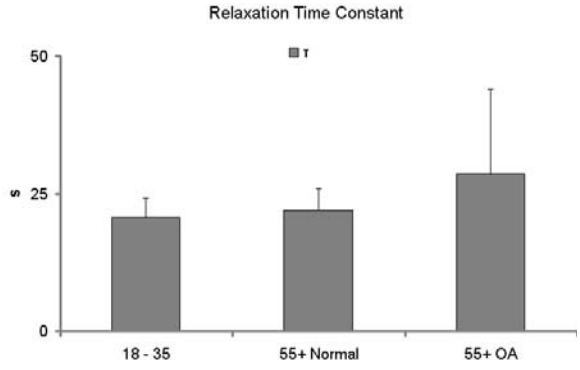
A



B



C



D

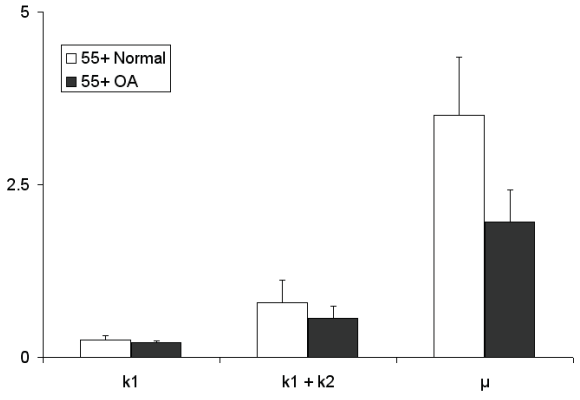


Figure 4: Viscoelastic parameters were significantly different between 18 – 35 and the 55+ groups. A: Equilibrium modulus (k1, p = 0.001); B: Instantaneous modulus (k1+k2, p = 0.05); and C: Apparent viscosity (μ, p = 0.03). No significant difference was found in relaxation time constant (τ) between groups (D). No significant differences were found between the Normal and OA subgroups of the 55+ group, and no significant differences were found between the cells isolated from Normal and OA tissue from the same donors (E).

26). In chondrocytes, several reports have shown that a viscoelastic solid model is more appropriate with very good approximation of experimental data (21, 22). Further, the viscoelastic solid model involves simple calculations for determining the biomechanical properties of the cell. We therefore used a previously described viscoelastic half-space model to study aging-related changes in chondrocytes (12, 21).

We found several differences with age. Cells from the 55+ group were significantly larger than those from the 18 – 35 group. The instantaneous modulus, equilibrium modulus, and apparent viscosity were all significantly higher in the 55+ age group relative to the 18 – 35 age group. Actin and vimentin have been implicated as major contributors to these viscoelastic properties of chondrocytes (10). Disrupting tubulin microtubules had no effect on cellular mechanical properties while disruption of actin and vimentin significantly reduced the instantaneous and equilibrium moduli as well as the apparent viscosity. Actin microfilaments are substantially stiffer than vimentin intermediate filaments under low stress or strain conditions (27). Actin microfilaments rupture at approximately 20% strain, while vimentin filaments show strain hardening and remain intact up to 80% strain. Both actin and vimentin polymers are viscoelastic but vimentin creeps substantially more than actin with a pronounced viscous flow behavior (27, 28). Taken together, these complementary viscoelastic properties indicate a composite material behavior. Therefore, more experimental evidence is required to establish whether actin or vimentin may be affected with aging.

The instantaneous modulus (k1 + k2) was similar between our study and that previously reported for non-arthritic chondrocytes (21). However, the equilibrium modulus (k1) measured for the 18 – 35 age group in our study was lower than but within the range previously reported (10, 21). These differences could be due to the differences in the source and preparation of the chondrocytes. Cells studied in the present study were harvested from knee articular cartilage and tested within 2 hours of digestion, whereas the previous study obtained cells from the hip and knee joint and precultured the cells in alginate beads.

We were unable to find significant differences in chondrocytes based on the grade of OA. Mean cell size measured for the 18 – 35 group (13.8 μm) was similar to that reported for non-osteoarthritic chondrocytes (13.7 μm) (21). However, we found a more significant increase in cell size with age ($p < 0.001$) than with OA (p = 0.06). One study found chondrocytes obtained from osteoarthritic tissue to be stiffer and more viscous than those obtained from normal cartilage (21). On the other hand, another study reported no differences in the elastic modulus of normal and arthritic chondrocytes (22).

By definition, Grade III OA involves partial-thickness loss of cartilage. Therefore, it is possible that the zonal distribution of cells obtained from normal tissue were not comparable to the cell populations obtained from Grade III OA cartilage and may contribute to apparent differences

in pooled cell properties. However, chondrocytes from the superficial zone are stiffer under atomic force microscopy (29). Therefore, loss of the superficial zone in the 55+ group would not by itself explain the increase in mean stiffness.

One weakness of the study was the potential for selection bias of cells that would survive the isolation procedure. To minimize the effect of cell division and time in culture, we conducted the micropipette aspiration experiments on freshly isolated cells (within 2 hours). Suspending chondrocytes in an isotonic solution may induce artifacts due to the loss of the pericellular matrix. Another weakness in our study design was the lack of donors with Grade III or IV OA in the 18 – 35 group. Including these donors would have helped establish the relative contribution of OA and aging to chondrocyte stiffness. However, it was extremely difficult to obtain cartilage from donors with established OA in the 18 – 35 age group.

These results suggest that aging is associated with cell stiffening. The likely mechanisms could be altered mechanical properties of the cell membrane, the cytoplasm, or the cytoskeleton. All of these mechanisms can affect the membrane aspiration length during micropipette experiments. The increased stiffness of osteoarthritic chondrocytes has been attributed to the actin cytoskeleton. It is possible that a similar mechanism is responsible for the increased stiffness found in aging chondrocytes. Other potential causes of increased stiffness include aging related intracellular protein changes such as oxidation, crosslinking, amino acid racemization or nonenzymatic glycation. With advancing age changes in the biomechanical properties of the cell could alter mechanotransduction pathways, and molecular and biochemical responses.

Acknowledgement: We are grateful to Farshid Guilak, PhD for assistance with the micropipette technique and data interpretation.

References

1. Olson, S. A. & Marsh, J. L. (2004) Clinical orthopaedics and related research, 2.
2. Barbero, A., Grogan, S., Schafer, D., Heberer, M., Mainil-Varlet, P., & Martin, I. (2004) Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society 12, 476-484.
3. Guerne, P. A., Blanco, F., Kaelin, A., Desgeorges, A., & Lotz, M. (1995) Arthritis and rheumatism 38, 960-968.
4. Loeser, R. F., Shanker, G., Carlson, C. S., Gardin, J. F., Shelton, B. J., & Sonntag, W. E. (2000) Arthritis and rheumatism 43, 2110-2120.
5. Martin, J. A., Ellerbroek, S. M., & Buckwalter, J. A. (1997) J Orthop Res 15, 491-498.
6. Messai, H., Duchossoy, Y., Khatib, A. M., Panasyuk, A., & Mitrovic, D. R. (2000) Mech Ageing Dev 115, 21-37.
7. DeGroot, J., Verzijl, N., Bank, R. A., Lafeber, F. P., Bijlsma, J. W., & TeKoppele, J. M. (1999) Arthritis and rheumatism 42, 1003-1009.
8. Brand, H. S., de Koning, M. H., van Kampen, G. P., & van der Korst, J. K. (1991) J Rheumatol 18, 599-605.
9. Livne, E. (1994) Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society 2, 187-197.
10. Trickey, W. R., Vail, T. P., & Guilak, F. (2004) J Orthop Res 22, 131-139.
11. Ingber, D. E., Prusty, D., Sun, Z., Betensky, H., & Wang, N. (1995) Journal of biomechanics 28, 1471-1484.
12. Sato, M., Theret, D. P., Wheeler, L. T., Ohshima, N., & Nerem, R. M. (1990) Journal of biomechanical engineering 112, 263-268.
13. Wang, N., Butler, J. P., & Ingber, D. E. (1993) Science 260, 1124-1127.
14. Heath, J. P. & Holifield, B. F. (1991) Cell Motil Cytoskeleton 18, 245-257.
15. Janmey, P. A. (1998) Physiol Rev 78, 763-781.

16. Riezman, H., Munn, A., Geli, M. I., & Hicke, L. (1996) Experientia 52, 1033-1041.

17. Sims, J. R., Karp, S., & Ingber, D. E. (1992) J Cell Sci 103 (Pt 4), 1215-1222.

18. Simon, V. R. & Pon, L. A. (1996) Experientia 52, 1117-1122.

19. Turner, C. E. & Burridge, K. (1991) Curr Opin Cell Biol 3, 849-853.

20. Hochmuth, R. M. (2000) Journal of biomechanics 33, 15-22.

21. Trickey, W. R., Lee, G. M., & Guilak, F. (2000) J Orthop Res 18, 891-898.

22. Jones, W. R., Ting-Beall, H. P., Lee, G. M., Kelley, S. S., Hochmuth, R. M., & Guilak, F. (1999) Journal of biomechanics 32, 119-127.

23. Evans, E. & Yeung, A. (1989) Biophys J 56, 151-160.

24. Needham, D. & Hochmuth, R. M. (1990) Journal of biomechanical engineering 112, 269-276.

25. Dong, C. & Skalak, R. (1992) J Theor Biol 158, 173-193.

26. Theret, D. P., Levesque, M. J., Sato, M., Nerem, R. M., & Wheeler, L. T. (1988) Journal of biomechanical engineering 110, 190-199.

27. Janmey, P. A., Euteneuer, U., Traub, P., & Schliwa, M. (1991) J Cell Biol 113, 155-160.

28. Janmey, P. A., Hvidt, S., Peetermans, J., Lamb, J., Ferry, J. D., & Stossel, T. P. (1988) Biochemistry 27, 8218-8227.

29. Darling, E. M., Zauscher, S., & Guilak, F. (2006) Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society 14, 571-579.