Biomechanics and MechanoBiology of Human Cartilage Articulation

Felix H Hsu[1], Eloy Alonso,[1] Aimee R Raleigh,[1] Abdulelah A Saleh,[1]
Koichi Masuda,[1] Martin K Lotz,[2] Albert C Chen,[1] Robert L Sah[1]
[1]University of California-San Diego, La Jolla, CA [2]The Scripps Research Institute, La Jolla, CA
Disclosures: FHH (N), EA (N), ARR (N), AAS (N), KM (N), MKL (N), ACC (N), RLS (N)

INTRODUCTION: During joint articulation, cartilage, particularly that near the articular surface, undergoes a complex combination of compression, shear, and sliding.[1,2] In vitro analyses have focused on cartilage biomechanics in response to relative surface movement at a constant velocity after startup. However, joint movement typically involves variability in the relative surface velocity. Cartilage also exhibits a variety of biological responses to shear and articulation, including increased matrix and lubricant secretion at low amplitudes but cell death at high amplitudes.[3,4] Chondrocyte death and survival generally involves mechanisms of apoptosis and autophagy, with high amplitude torsional shear causing cell apoptosis and mechanical impact attenuating autophagy but stress-related responses to articulation are unclear.[4,5] Microtubule-associated protein 1 light chain (LC3) correlates with autophagosome formation and PARP-p85 is a nuclear enzyme involved in DNA repair.[6,7] The objective of the present study was to assess the biomechanics and mechanobiology of articular cartilage when subjected to time-varying articulation in the form of low and high amplitude oscillations.

METHODS: ***Harvesting of human articular cartilage disks.*** Human articular cartilage disks (2 or 3 mm diameter, 1.1mm thick) were harvested to include the articular surface from the patellofemoral groove of 16 normal human knees (n=7 **Y**oung, <35 yrs, 27±5, range 19-34 yrs; n=9 **O**ld, >50yrs, 58±5, range 51-66 yrs), without evidence of osteoarthritis from cadaveric donors. Disks were incubated in medium supplemented with 10% FBS for 3-5 days, and then left free-swelling (**None**) or else subjected to ramp compression to 20% and then superimposition of ±100 μm (**Low**) or ±1000μm (**High**) at 1Hz sinusoidal lateral motion for 400 cycles with a polysulfone countersurface, using a chamber customized for a mechanical tester (Biomomentum).[8] **(1) Biomechanics. A**xial and lateral displacement and load were recorded, fit using Fast Fourier Transforms, and total harmonic distortion (THD) were computed. Lateral stiffness magnitude and phase were computed and Lissajous curves were plotted. **(2) Mechanobiology.** Effects of loading on SZ cell viability were determined by Live/Dead staining and expression of PARP-p85 and LC3 by IHC. Samples were snap frozen just after articulation, immunostained with peroxidase detection, and digitized at 40X, and then analyzed as for positive and negative staining cells as a function of depth from the articular surface. The percentage of positive cells was determined for the top 100μm, the superficial zone (**SZ**), and the next 600μm, the middle/deep zones **(MDZ)**. ***Statistics.*** Data are summarized as mean ± SEM, with n = # of donor samples per condition. Percentage data were arcsine transformed to improve normality and homoscedasticity. The effects of mechanical stimulus (amplitude) and/or age were assessed by t-test or repeated measures ANOVA.

RESULTS: (1) Biomechanics. Multi-axial articulation biomechanics showed characteristic amplitude-dependent features (Fig. 1). Lateral stiffness amplitude was higher at **Low** than **High** oscillation ($p < 0.005$, **Fig. 1A**), while stiffness phase was lower ($p < 0.001$, **Fig. 1B**); neither varied with age. Lissajous stiffness plots for **Low** oscillation were symmetrical steep ovals, while those for **High** oscillation were irregular shapes, with steep ascending and descending components just after the reversal of movement, and flattened regions during sustained movement associated with increased THD (**Fig. 1C**).
(2) Mechanobiology. Chondrocytes within cartilage exhibited apoptosis and autophagy responses to articulation **(Fig. 2).** LC3 expression was stimulated by **High** articulation in the **SZ** ($p<0.001$) with PARP-p85 exhibiting a similar tendency in **High** articulation-induced stimulation ($p=0.09$). In the **MDZ** cartilage, a tendency ($p=0.10$) of higher LC3 expression was observed in young compared to old chondrocytes, and in contrast, an age-dependent trend of articulation-induced PARP-p85 ($p=0.091$) expression, which was higher in old than in young chondrocytes.

DISCUSSION: The biomechanical analysis of articulation revealed **Low** amplitude responses consistent with shearing of the cartilage with minimal sliding and little energy dissipation, and **High** amplitude responses consistent with shearing and then sliding with substantial energy dissipation. In addition, variations in lateral load may represent stick-slip phenomena, associated with increased friction at lower sliding velocities. Articulation induced age-dependent responses of apoptosis and autophagy in human cartilage that may dictate chondrocyte phenotype and survival. Responses may vary as a function of time after loading. A detailed and combined understanding of mechanical articulation in tissue behavior and associated biological pathways and responses may clarify mechanisms of aging-associated deterioration of human articular cartilage.

SIGNIFICANCE: The multi-axial biomechanics of cartilage articulation can be analyzed using oscillatory signals with consideration and interpretation of magnitude, phase, and harmonic distortion. The mechanobiology of cartilage articulation at High amplitude involves age-associated apoptosis and autophagy responses that are substantially different than those involved in impact-associated damage.

REFERENCES: [1] KozanekM+, JBiomech 42:1877, 2009. [2] WongB+, ArthritisRheum 58:2065, 2008. [3] NugentG+, ArthritisRheum 54:1888, 2006. [4] Waller+, ProcNatlAcadSciUSA 110:5852, 2013. [5] CaramesB+, ArthritisRheum 64:1182, 2012. [6] KabeyaY+, EMBOJ 19:5720, 2000. [7] O'BrienM+, Biotechniques 30:886, 2001. [8] HsuF+, Trans Ortho Res Soc 40:256, 2015.

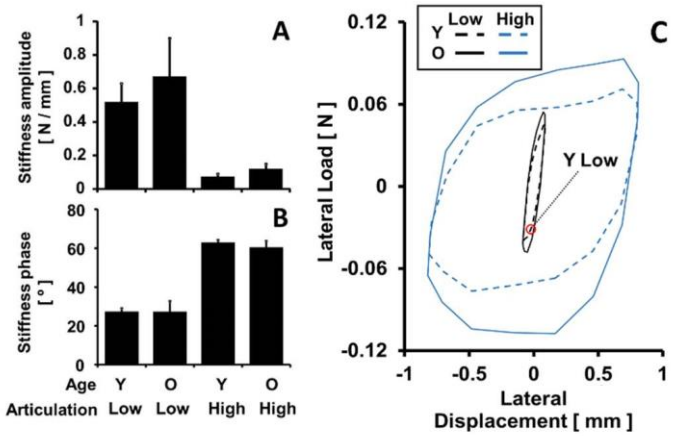
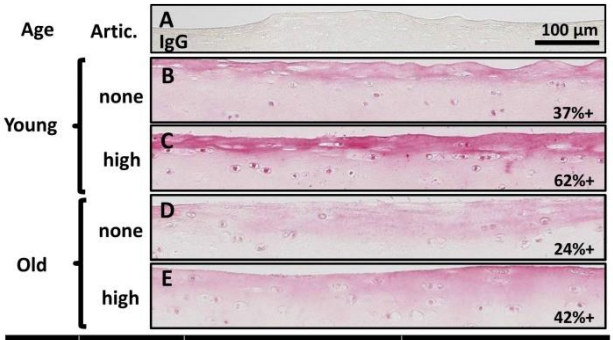


Fig. 1. The effects of oscillation amplitude and age on articulation biomechanics. Lateral stiffness **(A)** amplitude and **(B)** phase, mean ± SEM, n=5/grp. **(C)** Lateral load versus displacement for representative samples.



Donor Age	Articulation	%PARP-p85+		%LC3+	
		SZ	MDZ	SZ	MDZ
Young	none	41 ± 7	34 ± 11	25 ± 14	39 ± 15
Young	high	48 ± 4	30 ± 5	54 ± 4	34 ± 9
Old	none	32 ± 11	19 ± 7	16 ± 5	14 ± 5
Old	high	66 ± 6	34 ± 10	45 ± 2	29 ± 10

Fig. 2 and Table 1. Effects of high articulation on chondrocyte LC3 expression in SZ (A-E) and MDZ, and PARP-p85 expression in SZ and MDZ cartilage (table). mean ± SEM, n=4/grp.