A MULTIFREQUENCY SPECTROMETER FOR FAST ASSESSMENT OF THE DYNAMIC PROPERTIES OF CARTILAGE

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Introduction
The ability of cartilage to transmit or withstand loads depends on the integrity of its extracellular matrix [3, 6]. This ability is commonly defined by its dynamic stiffness and phase-shift [3]. The spectra of dynamic characteristics provide useful insights into matrix integrity and its resistance to damage [5]. Traditional testing using single harmonic waveform to determine the dynamic properties of cartilage [3, 7] was a time-consuming procedure. With a multi-frequency waveform, the total testing time, and thus risk of contamination, can be significantly reduced.Benefiting from modern data acquisition technique, we designed a spectrometer using the multi-frequency waveform (0.1-200 Hz) as an input for fast assessment of the dynamic properties of normal and degenerative cartilage.

Materials and Methods
Normal cartilage was excised from the skeletally mature humeral joint with a 4 mm biopsy punch. Cartilage disks was subjected to repetitive impacts 5MPa of at 0.3 Hz with 30% loading cycle for 120min (previously described in Farquhar et al., 1996). Cartilage with osteoarthritic lesion was obtained from two-year-old canine dysplastic hips. A spectrometer was designed to characterize the matrix changes in the a wide spectrum of frequencies (0.1-200 Hz). System consisted of amplifier, signal conditioning system, electrodynamic vibrator, and a specially designed loading frame (Fig. 1) with resolution of 0.2µm and 0.01N. Facilitated with inboard control, this spectrometer was capable to test tissue with multiple frequencies or user-defined waveforms. Load control and post-testing analyses were achieved with a commercially available programmable software, LabVIEW 4.1 (National Instruments), operating on a PowerMacintosh 8100/80 computer. Explant was subjected to a multi-frequency (0.1-200Hz) diagnosis waveform. The total diagnosis time was less than 30 sec to minimize possible loading stimulation. The maximum stress, 0.1 MPa, was within the linear region of stress-strain curve. Dynamic moduli and phase-shift at each frequency were calculated from the responding stress and strain after decomposition with fast Fourier Transform. Compositional analyses included water content, hypotonic swelling, and GAG content (via DMMB assay). Non-paired Student’s t-test was perform to determine the statistical significance (P<0.05).

Results
Results were highly repeatable (R²>99%) and consistent with single harmonic loads (R²>95%). Dynamic properties of degenerative tissues were found to be significantly different from normal tissues. Phase-shift of injured explant was increased by 0.6-1.1º while the dynamic stiffness (30%) was decreased (Fig. 2A). Phase-shift of OA lesion had higher increases (4.1-1.3º), and dynamic moduli were decreased 76%. A hypotonic swelling (4.6%), increase of water content (9.2%), and decrease of GAG content (Fig 2B) were found in the OA tissues. But, the hypotonic swelling and GAG content of impact-injured cartilage was not significantly different from normal.

Discussion
This study showed that using the multiple frequency waveform as an input significantly reduced the total time of testing, and thus the risk of contamination. This technique can potentially be applied to a servo-hydraulic system. Degenerative cartilage showed the low dynamic moduli and high phase-shifts that were consistent with compositional analyses. Since the maximum stress was in the linear region of strain-stress curve, the viscoelastic effect was minimal and decomposition was in good approximation [2]. This spectrometer is also small enough to operate inside an incubator, thus can monitor the progression of dynamic properties of in vitro injured cartilage.

References

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