Progress Report

2005 ISB

Student Dissertation Grant

Vibrational analysis of multiple stages of trabecular bone loss using rapid prototype duplicates

---

Wafa Tawackoli

Department of Bioengineering

Rice University

Purpose:

Osteoporosis is a systemic skeletal disease which increases bone fragility and susceptibility to fracture[2,3,6,7]. Osteoporosis afflicts about millions of people around the world and osteoporotic fractures are estimated to be several millions annually and cost tens of billions of dollars [2,10,13]. About 70% of these fractures are vertebral compression fractures, which often result in prolonged disability[10]. Characterization of bone quality in osteoporotic patients is currently very limited. Systems like DEXA (dual energy X-ray absorptiometry) neglect volumetric information of the scanned bone, and volumetric scanning systems, like quantitative computed tomography (QCT), are unable to detect disease related morphological deterioration due to image resolution and radiation exposure. A more direct approach is needed to reliably measure bone integrity and bone biomechanical properties, and detect possible structural complications. While some technical hurdles in developing a diagnostic tool using low frequency vibration have been overcome, many questions still remain including data interpretation and analysis. In particular, changes in the acoustic signal of bone have not been investigated at the various bone organizational levels.

Hypotheses:

In engineering applications, structural dynamics has been used extensively for micro-crack and damage detection[14]. I will explore the feasibility of using structural dynamics as a diagnostic tool for determining bone integrity. ***My principal hypothesis is that the vibrational modes of bone tissue changes significantly with the deterioration of bone micro-architecture and that these modes can be captured by sensors to infer a structural compromise.***

Long-term vision of clinical implementation:

The motivation behind this project was to prevent osteoporotic fractures (disuse and age-related osteoporosis) through early and reliable prediction of changes in biomechanical properties of bone. Such early detection may aid in the development of effective countermeasures to prevent bone micro-architectural deterioration and subsequent bone damage. Our work will determine if a direct method of determining biomechanical properties of bone tissue using structural dynamics can provide a more detailed profile of bone damage than standard clinical methods such as DEXA and QCT. Methods developed will also find application in the management of disuse-related osteoporosis.

Rationale:

I examined how the micro-architectural deterioration of bone tissue, due to osteoporosis, may affect vibration and dynamic responses on the bone and rapid prototyping duplicates (based on controlled computer models). It is not feasible to monitor the progression of osteoporosis on human trabecular bone in an acceptable time frame. In addition, if multiple samples with different density values are utilized, the total sample size would become astronomical, requiring representative sample size for each disease stage. Computer and rapid prototyping models allow for modifications of the

same sample, thereby reducing the number of samples required for these analyses. The biomechanical integrity of trabecular bone tissue depends on its material properties, but also on its microstructure.[4] In order to isolate the effect of structural changes, the material properties must be controlled; thus I used computer models and rapid prototyped duplicates of bone samples. A study of frequency response of different samples examines the differences in tissue volume, spatial distribution, and spatial orientation (morphology). I hypothesized that duplicate samples with the same density (volume fraction) but different micro-architecture will exhibit different frequency responses, hence different biomechanical properties.

Experimental Design:

Given the limitations and the impracticality of monitoring spinal loading at the site, all tasks were simulated under laboratory conditions. Several investigators[11,18] have shown that biomechanical experiments in a laboratory setting are comparable to *in vivo* measurements. In this study, cubic bone specimens from a single vertebra were prepared from the lumbar region of human male cadaver. To test my hypothesis, I selected a 0.05 level of significance, with a 90% chance of detecting a true difference between the measurements (structural dynamics, image regression) using a one factor ANOVA for each specimen group. Data obtained from the literature[17] on assessment of directional elastic modulus of ewe vertebral trabecular bone showed that the vibrational modulus correlated well with the elastic modulus measured in compression (r^2 = 0.72, n = 22). Using values from that study, and assuming acceptable type I and II errors of 5% and 10% respectively in combination with a one factor ANOVA power analysis, leads to a sample size of 5. For each bone cube, four computer models (Normal, Osteopenia, moderate Osteoporosis, and sever Osteoporosis) and one rapid prototyped duplicate (RPD) for each model will be generated. This will bring the total number of duplicates to 20 (5 levels x 4 models x 1 RPD).

Method:

L1-L5 vertebral bodies, from a human cadaver, was selected for coring of trabecular bone specimen (n = 10, two from each VB); only 5 samples were selected for my experiments. This sample size enables me to test for additional possible effects, such as geometric size of the bone by covariance analysis. Before preparation, X-rays were taken from the spine and will be reviewed by a clinical faculty colleague. The specimens were all classified as "normal"[5]

Fabricating Bone Models:

Rapid prototype system was used to fabricate scaled versions (10:1) of all 20 computer models (figure 1). Only twelve duplicates have been made up to this point. I performed an auxiliary study on the resonance frequency on rapid prototyped bone cubes. μCT scans of the lateral aspect of a human lumber vertebra of a 65 years old male was obtained on a μCT81 (μCT 81, Scanco Medical,

Switzerland) with an isotropic resolution of 30 μm. Through image manipulation a stereolithography (**stl**) file containing the triangulated surface of each bone cube was generated. An already available custom written algorithm was used to reduce the overall bone mass of the meshes through surface erosion. At the end of the simulation, reduction in bone mass separated the samples in four groups[5]; normal group (above 100 mg/cm^3), osteopenia (85-99 mg/cm^3), moderate osteoporosis (below 75-85 mg/cm^3), and severe osteoporosis (below 75 mg/cm^3), addressing the timeline and progression of osteoporosis and its effects on bone biomechanical properties. The overall bone mass of the normal bone cube was reduced through surface erosion to an osteopenic stage with BMD of 89 mg/cm^3, to a moderate osteoporotic stage with BMD of 78 mg/cm^3, to a sever osteoporotic stage with BMD of 70 mg/cm^3; this procedure influenced bone volume fraction as well as connectivity. After scaling up both models to a 12 cm edge length, digital files were exported to a rapid prototyping system for fabrication. The system currently used for this project was a 3-D phase change printer (ZPrinter 310, Z Corporation, Burlington MA). After fabrication, the normal, osteopenic, moderate osteoporotic, and sever osteoporotic bone model weighted 400, 328, 272, and 254 grams, respectively. To prepare for vibrational testing, both top and bottom of the bone models were fixed in cast cement. The actual construct weight for each bone model prior to each test is shown in table 1.

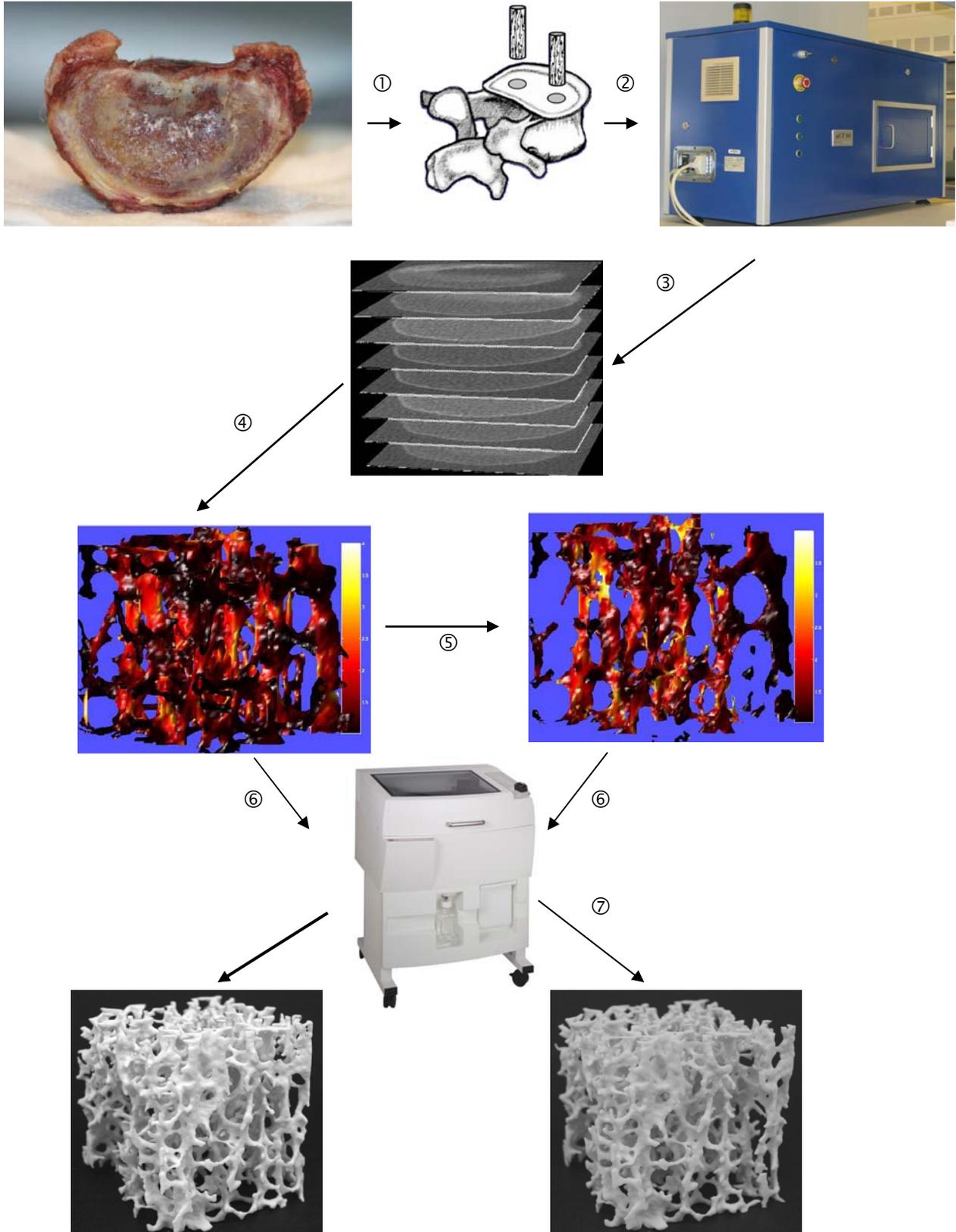| Bone Models	Weight [g]	Plate	Main Bolt	Small Bolts	Actual weight	Wax
Normal	400.87	118.60	7.71	3.40	554.11	23.53
Osteopenic	328.14	118.11	7.66	3.41	491.29	33.98
Moderate Osteoporotic	272.15	119.12	7.92	3.42	434.32	31.87
Sever Osteoporotic	254.14	118.05	7.61	3.39	416.97	33.77

Figure 1: **Fabricating Bone Models**. 1) A healthy single vertebral body from lumbar region was prepared , 2) A cylindrical core (8 mm x 20 mm) was extracted from the vertebral body, 3) microCT scan was taken from the bone core, 4) Triangulated surfaces were generated from a 6x6x6 mm bone cube within the scanned cylindrical core, 5) Bone mass was reduced through surface erosion to mimic osteopenic stage, 6) Digital files were exported to a rapid prototyping system, and 7) scaled version of a normal (left) and osteopenic (right) bone cubes were made.

Vibrational Analysis [3,4]:

With the help of my advisor, I have developed a device (*OseoSonic*) and control/measurement software that can apply mechanical vibrations to bone tissue *in vitro* and *in vivo* at various frequencies and intensities for structural research and modal testing. The *OsteoSonic* device produces a dynamic force operating on the mass reaction principle. A reaction force excites the structure under test. Both input signal (dynamic force) and output signals (acceleration/force) are measured at point contact to the test structure using an impedance head. The current device uses a single point of contact between the shaker and the test structure with a pre-determined contact pressure, which depends on anatomic site, bone shape, and surrounding soft tissue. The surrounding soft tissue does have a damping effect but does not alter the resonance frequency of the underlying bony structure[1,12,16]. Through this grant, I have upgraded the device to allow for two sites output signals and higher resolution data acquisition system which is essential for frequency studies. A pre-determined contact force of 50N (no bending moment, no shear forces) was applied during the experiment along the major loading axis. The compression force was monitored with a 6 DOF load cell. Vibrational analysis were carried out by applying a swept sine dynamic force (F1 = 300 Hz, F2 = 1300, Δf: 1 Hz) or white noise to each the normal and the osteopenic bone model cubes while simultaneously recording acceleration and dynamic force.

Dynamic responses of the bone replicas were recorded through an impedance head mounted between the sample and the *OsteoSonic (Figure 2).* These analyses allowed me to separate changes in sample dynamic response to the different types of micro-architectural deterioration. Similar vibrational analyses were performed on the bone cubes. Linear regression analysis on the results allows for comparison of the results between groups. This approach will quantify the sensitivity of the measurement systems to detect the changes in bone mass and determine bone quality using structural dynamics. Measures of three-dimensional morphology were obtained for each trabecular bone cube obtained from μCT images, which will help me later on to evaluate standard architecture parameters such as anisotropy ratio, Euler connectivity, mean trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular spacing (Tb.Sp)[15]. The sensitivity will be assessed by quantifying the shift of peak frequency of the first and second resonance modes.

The combination of μCT images (to obtain bone micro-architecture) and rapid prototyping (RP) (to fabricate samples with isotropic material properties) was used to fabricate scaled up models of bone. Consequences of architectural changes can be made. These types of model represent an idealized approach such that only allows architectural changes to affect the mechanical properties of the models.

LabView 7.1 was used to control the mechanical vibration and data collection. Two programs were written in LabView to control the mechanical vibration: White Noise and Swept Sinewave. Aluminum rods, stainless steel rod, plastic rod with different dimensions were used to calibrate the system. The

expected results were mathematically calculated. By changing the input variables (figure 6), the expected results were achieved for these controls for both the white noise and swept sinewave.

White Noise: Uniform random white noise was generated with 2000 Samples/second for a total of 3 minutes. The results permitted us to use frequency response function (FRF) to analyze data from 0 to 1000 Hz with 0.1 Hz resolution. The optimized stimulus amplitude was measured at 0.350 Volts. Data were collected from 3 to 4 channels (figure 3). RMS (root mean square) and exponential weighting were used. Exponential weighting emphasizes on new spectral data more than old ones. This technique strengthens the dynamic equilibrium and lowering the noise to signal ratio. Averaging takes place according to this formula in real time:

$$\text{New Average} = \text{New Spectra} * 1/N + \text{Old Average} * (N-1)/N$$

Swept Sine wave: The labview program generates a tone for the excitation signal and measures the root-mean-square (RMS) levels of response channel the magnitude of frequency response of the DUT. The measurements are performed at each test frequency, one frequency at a time. Sinewave signals were generated with 0.35 volts amplitude and 128 data point/cycles for about 50 cycles/freq. This amplitude was chosen since it was found to give the optimum results. Higher level produces noise and lower level does not produce detectable response. Frequency from 300 to 1300 [Hz] was applied with a resolution of 1 Hz. Raw data were collected from 3 to 4 channels (figure 3).
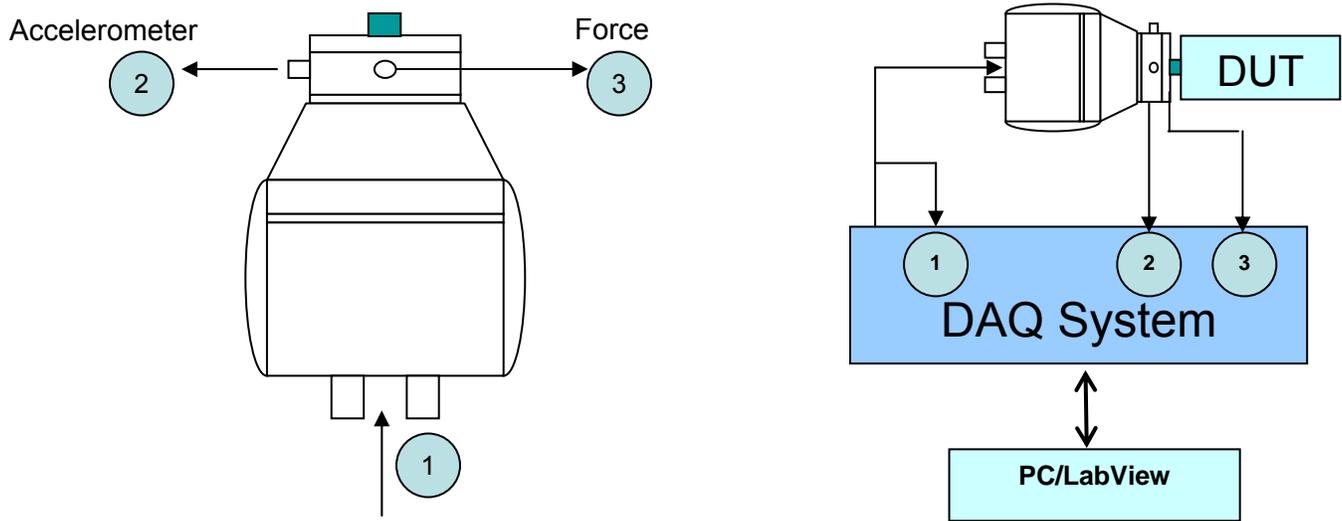
Figure 2: **Vibrational Setup.** A schematic diagram of vibrational testing system. (*left*) The Device Under Test (DUT) is vibrated under sinewave or white noise. There are three signals: (1) input, (2) acceleration, and (3) force. The signals are generated and acquired by LabView through a PXI-DAQ system connected to a PC. (*right*) Vibration testing setup. Bone models were attached to the vibrational testing device.
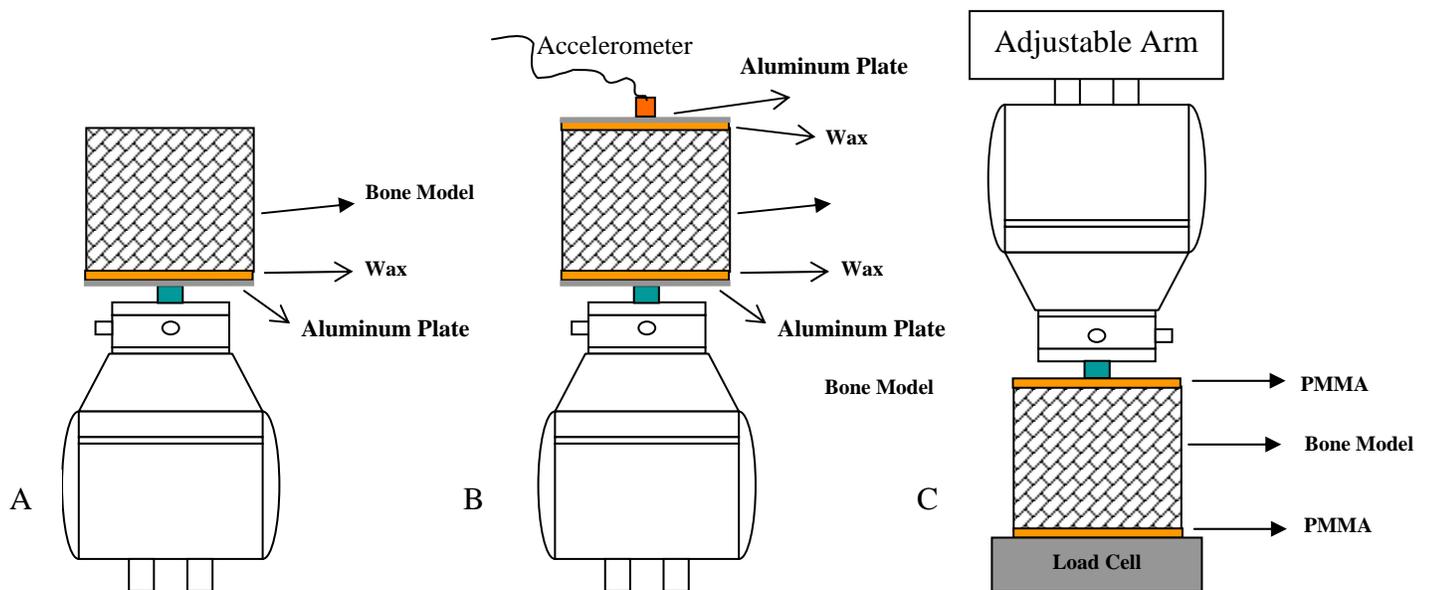

Figure 3: **Test Configuration.** (A) Direct measurement – wax and aluminum plate on one side only, (B) Direct and Indirect measurements – wax and aluminum plate on opposite sides + external accelerometer, and (C) Direct and confined compression measurement – wax and aluminum plate on opposite sides + external Load Cell. Static compression load of approximately ~50 [N] was applied to the bone model while testing.

***Limitations*:**

Osteoporosis affects bone mass and micro-architecture of bone and less the tissue composition. I therefore believe that my scaled rapid prototyped bone models displays the same relative behavior as a bone sample would if monitored over time. I do not expect the absolute values to be the same as material properties and scale size differ; however, the same trend can be expected as the mirco-architecture is identical. In addition, while the cubic shape of the specimen is beneficial for vibration testing, a cylindrical shape of bone specimens embedded in end-caps has been shown to minimize end-artifacts during mechanical testing to failure. Based on investigations by Keaveny and colleagues, I can compensate for the differences in experimental techniques provided that I perform experiments on cubic and cylindrical samples taken from the same vertebra and assuming sagittal symmetry.

Milestone:

- Extract human cadaver vertebral body-done (done)

- Scan vertebral body using MicroCT-done (done)

- Generate a bone cube within the scanned vertebral body using analyze-done (done)

- Convert files, increase size (15 times), and check connectivity of the bone model-done (done)

- Generate four induced osteoporosis models using the normal bone model (total of five computer bone models)-done (University of Houston-done)

- Check connectivity and prepare computer models (total of twelve[1]) for rapid prototyping-done (done)

- Generate physical model replicates from their computer models-done (60%)

- Mount bone models to plate using wax (60%)

- Apply different mechanical vibrational test to the bone models (50%)

- Data analysis (50%)

***Current Results*:**

The power spectra of the force and acceleration were computed. Velocity and displacement, dynamic stiffness, half-power bandwidth at peak, and damping ratio for both bone cubes were obtained using FRF measurements and mathematical integration over the frequency spectrum. Figure 4 and 5 are sample results from signal analysis.

The normal bone model showed the weakest modal coupling in power spectrum, whereas the sever osteoporotic bone model showed the strongest modal coupling with both models exhibiting a strong first peak at 350 Hz, 500 Hz and second peak at 1120 Hz, 675 Hz, respectively. The half-peak bandwidths for the normal and the osteopenic bone were determined to be 90 (ζ = 0.04) and 10 (ζ = 0.01) Hz for the second peak, respectively. The dynamic stiffness of the normal bone model was about five time of the osteopenic bone model. The osteopenic and moderate osteoporotic bone models have shown results between the two extremes bone models (Normal and Sever Osteoporotic). Although at a preliminary stage, the results have shown a clear difference

---

[1] The connectivity and thickness of the trabeculae in the last computer bone model was not sufficient enough for rapid prototyping.

between these four bone stages. Vibrational analysis in two additional axes will be performed and vibrational response sensitivity due to loading condition will be investigated. Further studies are currently underway to ensure the feasibility and reliability of a structural dynamics approach to detect early stages of bone loss.
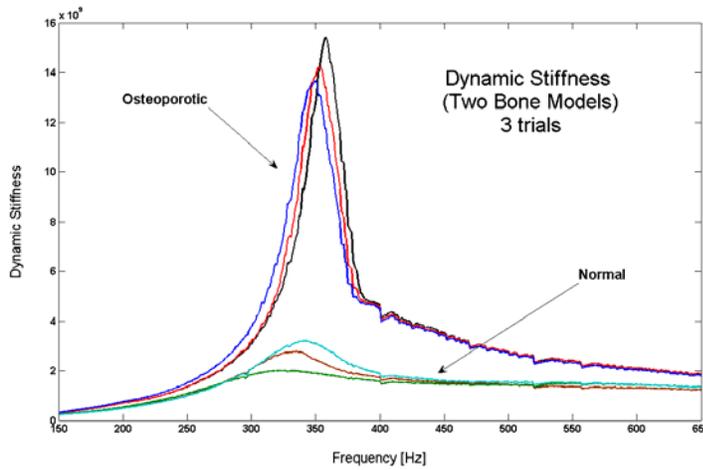

Figure 4: **Vibrational Results.** A comparison of dynamic stiffness of Normal vs. Osteoporotic bone model.
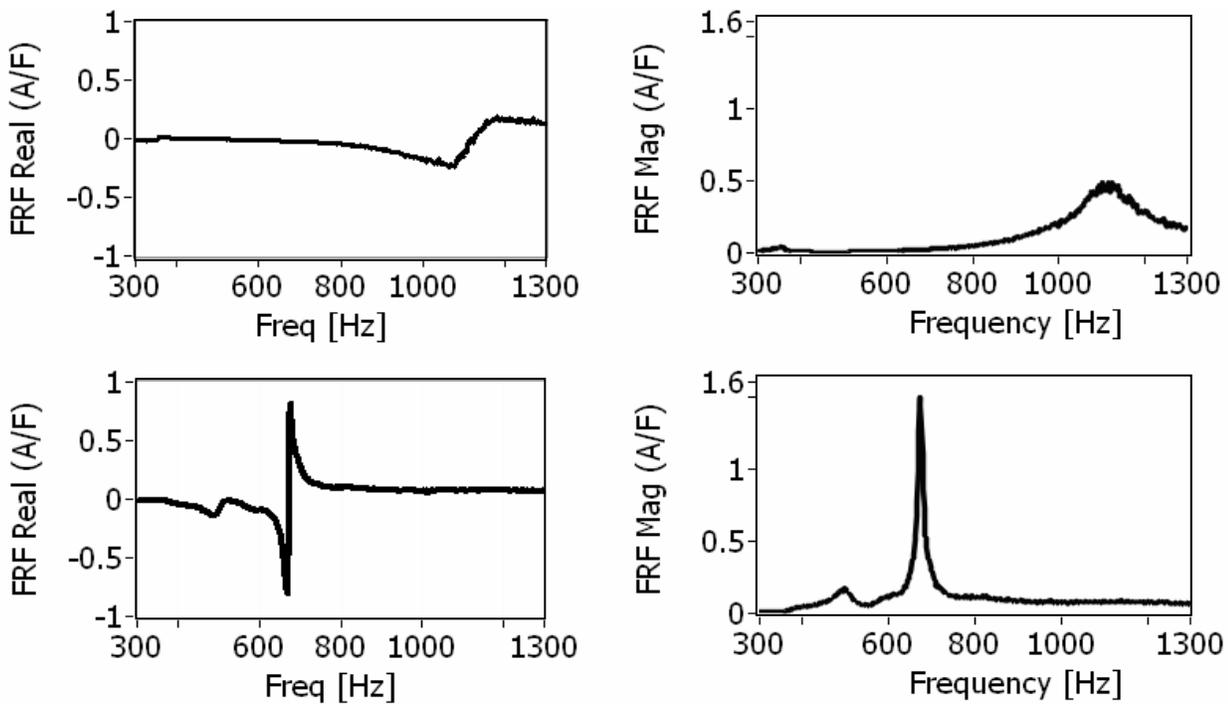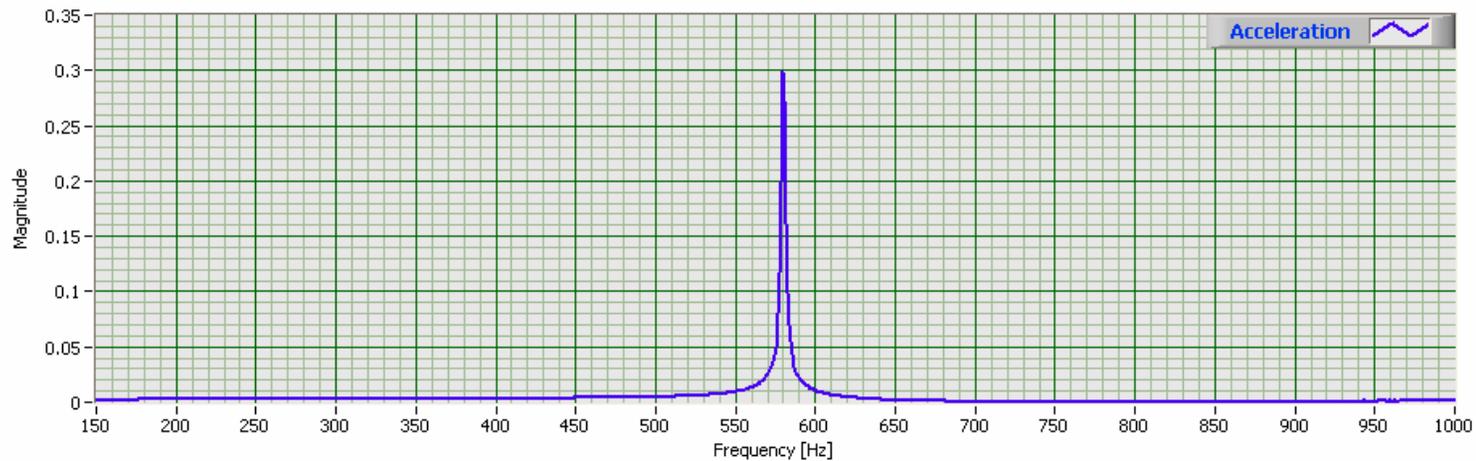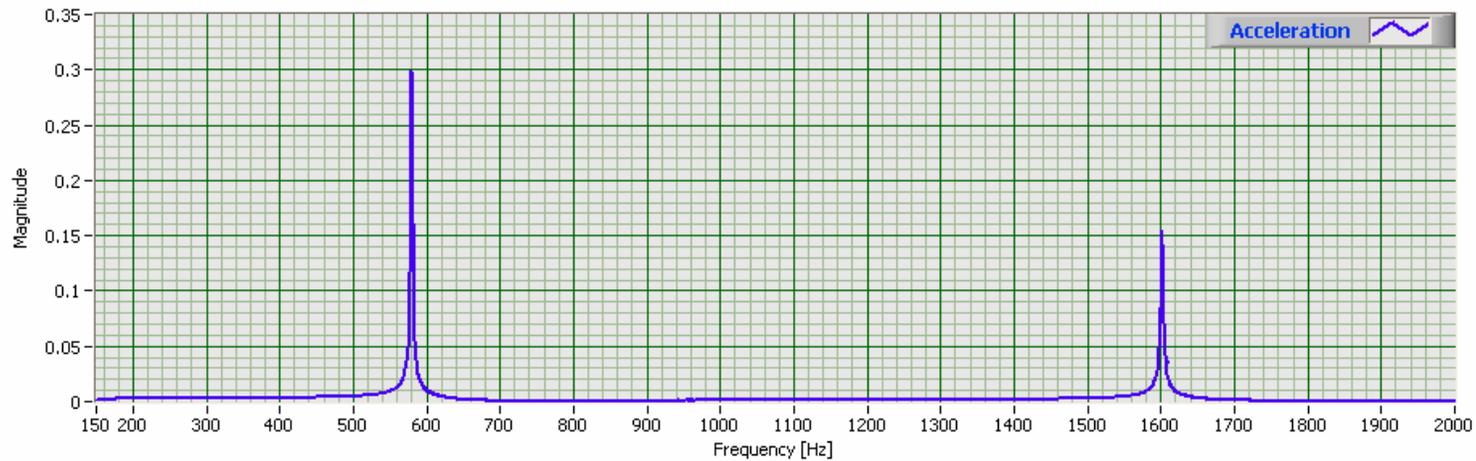

Figure 5: **Vibrational Results.** Real component (left) and magnitude (right) of inertance frequency response spectrums of normal (top) and osteoporatic (bottom) bone models.

The results form this study will reveal a trend within the dynamic signal to indicate the progression of osteoporosis at the trabecular bone architectural level. I expect this trend to be used later on patients as a more reliable diagnosis of osteoporosis. In addition, since spatial distribution of material can change mechanical properties by more than one order of magnitude, our analysis should indicate that micro-architectural

deterioration are more significant than bone mass loss. Future manuscripts will be submitted to the ISB-affiliated journals with full acknowledgement of ISB support in any publications, presentations, or posters.

Reference:

1. *Cornelissen P, Cornelissen M, Van der Perre G, et al. Assessment of tibial stiffness by vibration testing in situ--II. Influence of soft tissues, joints and fibula. J Biomech 1986;19:551-61.*
2. *Desai SS, Duncan BS, Sloan AS. The cost of treating osteoporosis in a managed health care organization. J Manag Care Pharm 2003;9:142-9.*
3. *Frost HM. Changing views about 'Osteoporoses' (a 1998 overview). Osteoporos Int 1999;10:345-52.*
4. *Gunaratne GH, Rajapaksa CS, Bassler KE, et al. Model for bone strength and osteoporotic fractures. Phys Rev Lett 2002;88:068101.*
5. *Hesch RD, Busch U, Prokop M, et al. Increase of vertebral density by combination therapy with pulsatile 1-38hPTH and sequential addition of calcitonin nasal spray in osteoporotic patients. Calcif Tissue Int 1989;44:176-80.*
6. *Kanis JA, Gluer CC. An update on the diagnosis and assessment of osteoporosis with densitometry. Committee of Scientific Advisors, International Osteoporosis Foundation. Osteoporos Int 2000;11:192-202.*
7. *Kannus P, Parkkari J, Sievanen H, et al. Epidemiology of hip fractures. Bone 1996;18:57S-63S.*
8. *Keaveny TM, Borchers RE, Gibson LJ, et al. Trabecular bone modulus and strength can depend on specimen geometry. J Biomech 1993;26:991-1000.*
9. *Keaveny TM, Pinilla TP, Crawford RP, et al. Systematic and random errors in compression testing of trabecular bone. J Orthop Res 1997;15:101-10.*
10. *Melton LJ, 3rd. Excess mortality following vertebral fracture. J Am Geriatr Soc 2000;48:338-9.*
11. *Miura T, Panjabi MM, Cripton PA. A method to simulate in vivo cervical spine kinematics using in vitro compressive preload. Spine 2002;27:43-8.*
12. *Nokes L, Fairclough JA, Mintowt-Czyz WJ, et al. Vibration analysis of human tibia: the effect of soft tissue on the output from skin-mounted accelerometers. J Biomed Eng 1984;6:223-6.*
13. *Norris RJ. Medical costs of osteoporosis. Bone 1992;13 Suppl 2:S11-6.*
14. *Pandey AK, Biswas M, Samman M. Damage Detection from Changes in Curvature Mode Shapes. Journal of Sound and Vibration 1991;Vol. 145:321-32.*
15. *Silva MJ, Gibson LJ. Modeling the mechanical behavior of vertebral trabecular bone: effects of age-related changes in microstructure. Bone 1997;21:191-9.*
16. *Steele CR, Zhou LJ, Guido D, et al. Noninvasive determination of ulnar stiffness from mechanical response--in vivo comparison of stiffness and bone mineral content in humans. J Biomech Eng 1988;110:87-96.*
17. *Weinhold PS, Roe SC, Gilbert JA, et al. Assessment of the directional elastic moduli of ewe vertebral cancellous bone by vibrational testing. Ann Biomed Eng 1999;27:103-10.*
18. *Wilke HJ, Rohlmann A, Neller S, et al. Is it possible to simulate physiologic loading conditions by applying pure moments? A comparison of in vivo and in vitro load components in an internal fixator. Spine 2001;26:636-42.*

Figure 6: **Control Panel.** (Top) Sample real time data analysis for acceleration (with limited capability during real time data collection). (Bottom) custom control panel allowing for changing variables in real time.

Budget Justification:

Funds were requested to improve and modify the existing DAQ and testing system. The previous system did not have the high-resolution and high-speed capability for performing full vibrational analysis. The new NI-DAQ system allows for 16-bit resolution and 500 kS/s, and has 16 analog inputs and 4 analog outputs (NI PXI-6229 M Series Multifunction DAQ). This new board was inserted into a high-power PXI chassis with reduced acoustic noise emissions (PXI-1031). The rapid prototyping service cost $900.00 (12 bone samples in total, $75.00 each). A fund was requested ($150.00) for mechanical and material items such as PMMA, adhesive materials, and plates (to hold the bone replicas). These materials were available to me from another laboratory and local machine shop for no additional cost. An additional fund was requested to upgrade miscellaneous electronic parts (i.e cables, connectors, accelerometers, power supply, and balance). The total fund requested was $4000.00 ($2000.00 dissertation fund and $2000.00 matching fund from my advisor). Expenses for cadaver specimens, μCT, X-rays, software license or any other typical cost associated with such projects have already been fulfilled. However, additional costs were accounted after compilation of the project such as delivery costs and a new adjustable arm to hold the vibrational equipment. Majority of these costs were paid by me and other sources.

Item #	Product Description	Parent Company	Store	New Price
1	Woodstock International H2572 10 pc. Needle Nose File Set	Woodstock International	Amazon.com	$ 7.50
2	Dremel 400-6/90 Series XPR Kit	Dremel	Amazon.com	$ 39.95
3	TL21 Minigrabber to Minigrabber IC Test Lead Set	Elenco Electronics Inc.	Amazon.com	$ 6.95
4	SL540 Assembled Soldering Station	Elenco Electronics Inc.	Amazon.com	$ 29.95
5	Extech EX430 True RMS Multimeter	Extech Instruments	Amazon.com	$ 39.99
6	Avenger 6" Caliper	Avenger Products	Amazon.com	$ 12.99
7	Bone Sample	Z-Corp		$ 900.00
			Total	**$ 1,037.33**
8	MXI-4 (Copper)/PXI 4-Slot Chassis/LabVIEW FDS Bundle (Academic)	National Instrument	National Instrument	$ 1,995.00
9	SHC68-68-EPM Noise Rejecting, Shielded Cable, 1 m	National Instrument	National Instrument	$ 60.00
10	CB-68LP Low-Cost, Unshielded I/O Connector Block	National Instrument	National Instrument	$ 63.00
11	NI PXI-6229 M Series Multifunction DAQ and NI-DAQ Software	National Instrument	National Instrument	$ 697.50
			Total	**$ 2,815.50**
12	0-50VDC/3A MASTECH METERED BENCH SUPPLY	MASTECH	MPJA	$ 99.00
			Total	**$ 99.00**
13	Ohaus SP-6000 Scout Pro Balance, 6000 g x 1 g	Ohaus	Scales Galore	$ 99.00
			Total	**$ 99.00**
			Overall Cost	**$4,050.83**