Bone Characterization Analysis

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Motivation

Mechanical behavior of bone is not yet fully understood due to bone's complex hierarchical structure. There is also little literature available on effects of maturation (aging) on the properties of bone (Fig. 1).

Bone consists of proteins (mainly collagen) and minerals (Fig. 2). By treating bone to separate its constituent phases, bone can be tested at a more fundamental level. Demineralization and deproteinization can be done to isolate the organic and inorganic phases respectively. This is a more recent approach and the effects of various chemical solutions are still being examined.

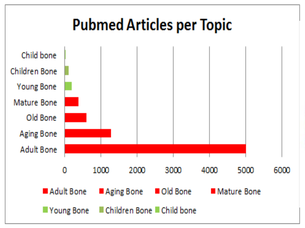


Figure 1: Chart comparing the number of studies done on bone at different ages.

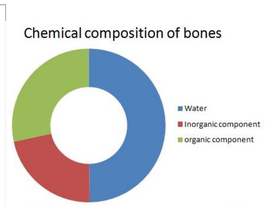


Figure 2: Relative chemical composition of bone.

Sample Preparation

Porcine femurs at specific ages were harvested from which mid-diaphysis bone (Fig. 3) samples were cut. Bones from 0, 4, 8, 12, 16, 20, and 24 weeks old pigs were used.

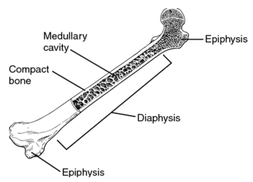


Figure 3: Bone specimens from the mid-diaphysis region were targeted.

Cross-sectional samples were prepared a few mm in height for Raman spectroscopy, and sectioned into smaller rectangular prisms for micro-CT and acoustic emissions studies (Fig. 4).

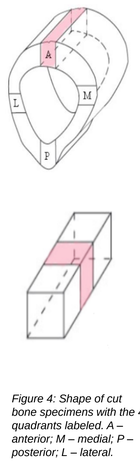


Figure 4: Shape of cut bone specimens with the 4 quadrants labeled. A – anterior; M – medial; P – posterior; L – lateral.

Demineralization was done through 0.1 M EDTA solution. Full demineralization takes about 4 weeks with daily replacement of the solution.

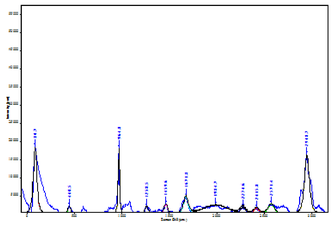
Deproteinization was done using 5.6 wt.% NaOCl solution. Full deproteinization takes about 2 weeks with daily replacement of the solution.

Method

- Samples were prepared as described and stored as 'wet' in .01M PBS solution (long-term) or 50/50 vol% ethanol/PBS solution (short-term)
- Samples were taken from the femur due to its larger size as compared to other regions such as the tibia or humerus
- Humerus samples were prepared for other future tests such bending and compression testing

Raman Spectroscopy

- Bone samples were prepared as described above
- Each bone sample was excited under strong concentrated light (laser, 785 nm)
- The laser causes molecular vibration of the object and emits photons of unique spectra
- By the emitted spectrum, relative molecular composition comparisons can be made
- Raman spectra can be mapped over a region
- Spectral data was collected and the area under the curve was analyzed



A 12 week Raman spectroscopy graph (Fig 5). Each of the peaks are marked, and they correspond to specific molecules.

The meaning of the peak can be determined by looking up shift values in previous studies (Table 1).

Figure 5: The resulting Raman spectroscopy graph measured on a 12 week old bone sample.

Porcine femurs of 0, 12, and 24 weeks have been studied. The peaks indicate Raman vibration at a corresponding Raman shift number. Each of the peaks represents a specific type of molecule and the height of the graph indicates the intensity, or the relative amount of a molecule (Fig. 6).
Same peaks across ages were analyzed because the peak locations do not change; it is only relevant to the type of molecule. The data is presented by comparing the relative intensities of the peaks. The area under the peak curve was measured and ratio of these values were calculated to make conclusions about the relative amount of molecules present.

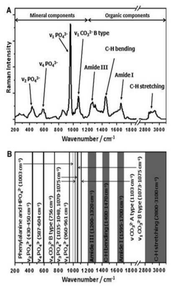


Figure 6: Bone spectroscopy and the peaks of interest.

Table 1: Raman shift numbers and month. Each molecules have specific Raman shift numbers. The slight differences can be seen across ages.

	Raman Shift (cm-1)		
	0 Month	12 Month	24 Month
PO43- v2	436.9	438.7	436.9
PO43- v4	594		
PO43- v1	964.8	964.8	964.8
Amide III	1257.45	1255.25	1255.3
CH2	1459.6	1458	1462.8
Amide I	1667.05	1670.2	1668.6
CH stretch	2944.7	2947.4	2947.4

Micro-CT

3 mm x 3 mm x 5 mm bone samples are being imaged through micro-CT at the Beckman Institute. Data is being processed.

Acoustic Emissions

Specimens with similar dimensions to those of the micro-CT prepared specimens are being tested in acoustic emissions studies at the University of Barcelona. Data is being processed.

Raman Results

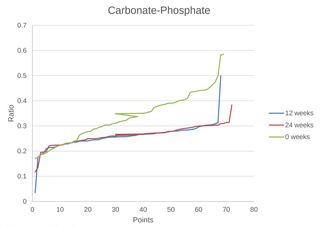


Figure 7: Carbonate-to-phosphate peak area ratio. Total of 70 points have been collected.

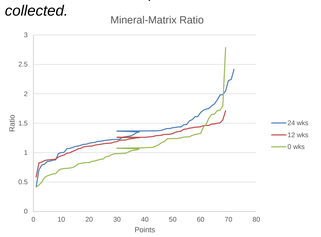


Figure 8: Phosphate-to-amide I peak area ratio. The ratio indicates mineral-matrix ratio.

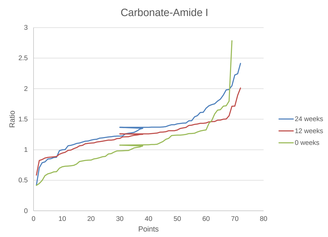


Figure 9: Carbonate-to-amide I peak area ratio.

A set of 21 point spectroscopy have been collected in each of the femur's anterior, posterior, medial, and lateral region, totaling up to 84 points in each bone sample. Figure 7 is the carbonate-to-phosphate ratio. High carbonate-to-phosphate ratio indicates highly fractured specimen. The resulting graph indicates that the younger bone has higher carbonate-to-phosphate ratio. This is not the expected result—older bones are more likely to fracture. The unexpected results may be due to the 0 week sample not maintaining its original composition due to its smaller size.

Figure 8 displays the mineral-to-matrix ratio. As bone age, the mineralization of bone generally increases. Our data indicates that there is a large difference between the mineralization of 0 weeks old specimen and 12 or 24 weeks old specimen. This indicates that there is a larger developmental gap between 0 weeks to 12 or 24 weeks.

Figure 9 shows that the carbonate-to-amide ratio are similar with 0 weeks sample having lower ratio. This ratio indicates bone remodeling.

Conclusions

Mineral-to-matrix ratio increases as age increases, which indicates that older bones are more mineralized. Carbonate-to-phosphate ratio of 12 and 24 weeks are similar and 0 weeks are significantly higher, which means there was more carbonate substitution in 0 week-old bone. Carbonate-to-amide I ratios also increased with age, and it indicates bone remodeling. Future improvement of this experiment include keeping the sample fresh and avoiding repeated thawing, as this may influence the spectrum.

Future Directions

- Repeat the Raman, micro-CT, and acoustic emissions tests and analysis with treated demineralized and deproteinized specimens
- Collect and analyse results from micro-CT and acoustic emissions tests
- Examine structural and material properties through ultrasound characterization, mechanical strength tests, scanning electron microscopy (SEM), and nano-indentation
- Study the effects of other chemical treatments such as the removal of collagen
- Test more intermediate age groups (between 0 and 12 weeks old and between 12 and 24 weeks old)

Acknowledgments

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