Development of a Mechanical System for Osteon Isolation

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Abstract
Osteons are small semi cylindrical hard tissues that exist in long bones of humans and some animals. Their diameter is in the range of 250 – 300 micrometers approximately. They contain the osteocytes which plays a role in bone mechanotransduction. The boundary that surrounds the osteon from outside is called the cement line. In a summer research project supported by CUNY-LSAMP a group of four students and three professors worked full summer to develop a system that can isolate the osteons thus a mechanical testing could be performed (stress relaxation test) to determine their poroelastic properties. Two sets of osteons are isolated; one contains the cement line and another one without the cement line (diameter less than 250 micrometers). The vision for the device is to be integrated into a microscopic system thus the osteons can be isolated while looking at them in the microscope. Therefore a new platform for the microscope is designed (Auto Desk Inventor software) and machined so that it can carry a hand drill that is free to move upward and downward. Drill bits are used to produce cylindrical samples of diameters of 200 and 450 micrometers thus allowing for isolation of the two sets of osteons. The drill is aligned with the microscope and two external deformable illuminators are used to give better microscopic images. The drill bits were aligned with the microscopic image in the same vertical plane. The isolated samples were tested by the Micro-CT (resolution is 1 micrometer) to check for any micro cracks that might have occurred during the isolation process. The results revealed that no micro cracks and the top and bottom surfaces are flat thus making the unconfined compression test [1] more accurate. A system that developed previously in the lab [2] is used to apply a displacement that is less than 30 nanometers in a stress relaxation test. The analytical approach [1] will be compared with the experiment results to obtain the poroelastic properties and the permeability of a single osteon in the presence and absence of the cement line.

Key words: Osteon Isolation, Lab innovation, Micromechanical Testing

Introduction

Any attempts to understand and model the biomechanical properties of bone must be firmly rooted in knowledge of the structure of bone on both the ultrastructural and microstructural levels of organization (Figure 1). Mechanical properties of bone are determined by a multiplicity of materials and structural properties such as tissue mineralization, size and composition of mineral crystals, anisotropy …etc. Time varying mechanical loads applied to bone generates fluid pressure gradients in the lacunar canalicular porosity (PLC) that contributes to the interstitial fluid flow and the transport in the PLC. The interstitial fluid flow is important for cellular nutrition and waste removal, and it is a critical factor in osteocyte mechanotransduction.

The osteon is composed of a central Haversian canal housing a blood vessel and is surrounded by alternating mineralized collagen lamellae as shown in Figure 1. The mean Haversian canal and osteon diameters in femoral bone are estimated to be 101±18 and

312±48μm respectively [3]. Their small size and stiffness make osteons challenging to isolate, nonetheless several approaches have been described in the literature since 1959. Ascenzi and coworkers,[4][5] [6] [7], developed a method for extraction of osteons from a thin cross-section of bone using a sharp steel needle eccentrically inserted in a dentist's drill or using a custom made micro lathe. Osteons have also been isolated by propagating a fracture through the natural boundary of the osteon [8] and using a precision and computer controlled osteon pushout micro-testing system [9].

The purpose of this study is to design a new system for isolation of better samples of osteons. The project is motivated by CUNY-LSAMP that gives the undergraduate students the opportunity to conduct a summer research with a faculty member in science, engineering, technology, or mathematics. The group consisted of 4 undergraduate students where 3 of them are working towards getting their associate degree in mechanical engineering technology and the fourth one is a senior student working towards getting a bachelor degree in mechanical engineering technology. The research work is done under supervision of 3 professors from the mechanical engineering and biomedical engineering departments.

Materials and Method

Students are required to use the skills they gained in their academic programs in manufacturing and design. They have to use different types of milling machines, diamond saw, and 3D design with Autodesk Inventor.

Osteons can be found in long bones of some animals and all humans. We received fresh sheep and cow bones from a meat market. For storage, the bones were kept frozen in a lab refrigerator. The size of the bone was reduced using the Delta Shopmaster vertical saw (3x1x1 cm). The bones had to be almost rectangular shaped so that easy thin slices can be made using a low speed circular diamond saw. The low speed diamond saw (Bueler Isomet Inc.) made thin and smooth slices of pieces of bones (1 mm in thickness). Water was used as a coolant during the cutting operation to prevent heating and dehydration of the sample, thereby retaining the mechanical properties of the bone (Figure 3). For storage, the bones and their slices are kept in a small container with water and placed in a refrigerator.

The first task is isolating the osteons while looking through a camera microscope (Nikon Eclipse TS100). A stable structure was needed to drill into the sliced bone (less than 1 mm). It is decided that a new platform is needed for the microscope which would hold the drill steady and at the same time would allow the light to pass through the bone showing the osteons. Using a CAD program, a three-dimensional drawing of the device is designed (Autodesk Inventor) and made . Aluminum brackets, a hard plastic, threaded screws, nuts, and washers are all sized and shaped using the Delta Shopmaster saw and a milling machine (Grizzlly Industrial, Inc.) to make the platform. Aluminum L shaped braces are used to hold the threaded support for the drill. The drill should be free to move in the Z–direction only, while the specimens can be moved in the x-y plane. After the platform is machined and put together with the microscope, the bones were ready to be drilled using the hand drill (Proxxon Inc.). Water is repeatedly dropped on the drilling site to keep the osteon's moist replicating bone conditions. In addition to that, extra lighting was provided by the MI-152 high intensity illuminator (Fiber-Lite) which made the osteons easy to spot.

Cuts are made using diamond core drill bits (UKAM Industrial, Valencia, CA). The outer diameters of the three drill bits are 2.0 mm, 1.5 mm, and 1.0 mm. To minimize the system vibration and prevent bending when drilling, the length of the drill bits is reduced.

From the data shown in Table 1, the cuts made from the 1.0 mm drill bit proved successful because they go isolate samples with less than 250 micrometers in diameter which is good enough to isolate the cement line.
The isolated samples are taken to the Micro-CT (SkyScan Corp., Belgium). The Micro-CT has the ability to take x-rays of small objects. The purpose of using this machine was for locate in micro-cracks that might have taken place during the isolation process of the samples. The fractures would interfere with the permeability tests making the samples useless. A cylindrical plastic rod was milled to hold the osteons in place when inside the Micro-CT. The scanning process takes about four hours. During this process, the Micro-CT is taking pictures at 360 degrees. After the scanning process, a three dimensional image is reconstructed and rendered from a data viewer software (NReacon). Using this program, the Haversian canal and other pathways for the capillaries were located. The results revealed no occurrence of micro-cracks in the isolation process, Figure 3, a result that make the osteons ready for testing in a loading system that is previously designed [2].

Conclusion

The system that is built proved to be successful. The one millimeter drill bit would produce sizes as small as 0.193 mm (Table 1). This is sufficient since the osteons are approximately 300-250 micrometers (0.3 mm) in diameter. Thus, the extraction of an osteon without the cement line for further testing is possible. Images from the Micro-CT show that there are no fractures after isolating the osteons; therefore the samples are ready for testing (Figure 3). On the other hand, the images show that there will be reasonable flatness for the upper and lower surfaces of the extracted samples which is very necessary for the unconfined compression testing. In addition to that, the developed system made better approximation for the location of the haversian canal to be in the center of the sample in order to satisfy the requirements of the analytical solution that we developed previously [1]. The design work is a very good training for undergraduate students who participated in this work. They gained more self-confidence doing the design of a real system. Students are able to apply what they learned in the classroom and gain more engineering skills, thus get prepared for a promising future in engineering research and education.

The project also established a link for multidisciplinary cooperation between the biomedical and mechanical engineering departments at City College of New York and the mechanical engineering technology department at New York City College of Technology. This mix of students and professors helped to achieve this work by bringing different experiences.

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Figures and Tables

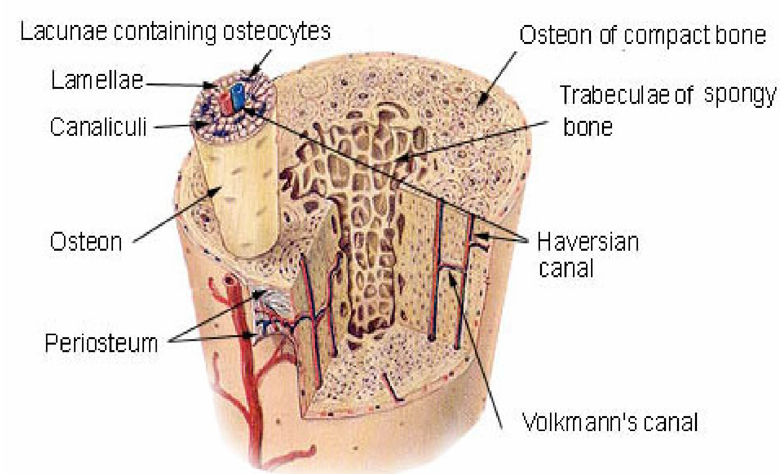

Figure 1: Sectional view of bone to show the osteons which is pushed out of the sample to show its details.

Figure 2: The full system for osteon extraction showing hand drill and deformable illuminators to improve the process of the isolation. The system is connected to a computer that can show the microstructure of the specimens and allow easy extraction of osteons.

Drill Bits outer diameter (mm)	Sample 1 Dia.	Sample 2 Dia.	Sample 3 Dia.	Sample 4 Dia.	Sample 5 Dia.	Sample 6 Dia.	Sample 7 Dia.	Sample 8 Dia.	Sample 9 Dia.	Sample 10 Dia.
2.0 mm	0.63	0.60	0.6	0.60	0.71	0.72	0.71	0.70	0.69	0.71
1.5 mm	0.53	0.40	0.44	0.50	0.47	0.46	0.46	0.45	0.46	0.45
1.0 mm	0.18	0.17	0.17	0.21	0.21	0.21	0.22	0.18	0.20	0.18

Table 1: Different sizes (Diameters in mm) of samples produced by different drill bits.

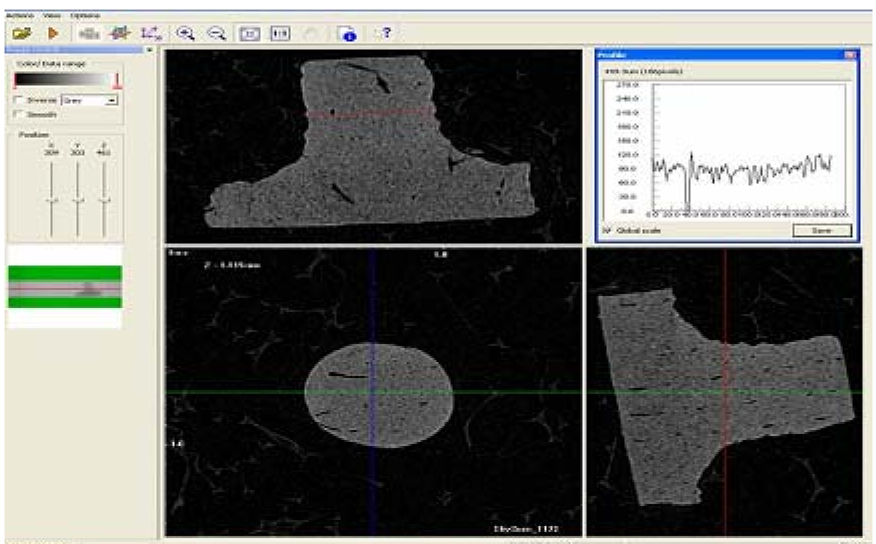

Figure 3: An image of one sample with diameter of 0.45mm after the reconstruction in the Micro-CT. the image shows that there are no micro cracks in the sample and the upper and lower surfaces are flat. The image is shown in Data Viewer software.