INTRODUCTION

Bones have a complicated structure built by layers of organic and inorganic elements. The composition and the arrangement of these materials give the bone its mechanical properties. The disruption of this matrix can lead to changes in the health condition of the bone. The natural frequency analysis is promising field of study which can give an indication about the health condition of the bone [1-3]. Inorganic materials (Calcium and Phosphate) are responsible for the bone strength [4]. Bone disease and calcium regulation can affect the bone density due to the change in the bone mineral density. Sometimes the bone is surrounded by an acidic medium due to the release of Lactic acid from the muscles in intensive exercise [5]. The acidic medium can also be generated due to the presence of metabolic acidosis which will influence the calcium from the bone. Patient who are suffering from osteoporosis take doses of calcium, but in many cases there is no significant change in the calcium content in the bones. The acidic medium may prevent the calcium placing inside the bone matrix and instead it will be used to neutralize this medium [6]. Many studies were done focusing on the effect of acidic medium on the bone composition and showed that osteoporosis and Metabolic Acidosis are linked [7].

The bone can be considered as a beam made of composite materials (organic and inorganic) and its mechanical properties depend on the nature of distribution of those materials. The disease and calcium regulation inside the body will affect directly the density of the bone. The mechanical properties of the bone have strong correlation with the apparent density. The structural dynamic properties of any structure depend on the mechanical as well as geometrical properties. If the bone is losing calcium due to any reason this will reduce its mass and the structural dynamic properties of the bone will change. Frequency analysis is a technique that can be used to verify the health condition of the bone [8]. All the standard test available to measure the bone integrity or to diagnose bone disease are radioactive. Therefore, developing a new diagnostic test without radiation hazard is highly important. To do so we need to understand how the natural frequency of a bone changes when the inorganic elements in the bone matrix and the natural frequency of the bone.

Finite Element Analysis

A finite element model of the bone is difficult to be built due to its shape irregularity and density variation. In order to build a three-dimensional model a CT-Scan test (Figure 1) was used. The images of the bones were collected and stored in DICOM format (Digital Imaging and Communications in Medicine). Those images have the size of 512 × 512 pixels. CT-scan device type GE Medical Systems\' Discovery CT 750 HD was used and the images were taken with the same setting (KVP 120, slice thickness 2.5 mm, and tube current 120 mA). The test was conducted before and after submerging in diluted acid.
Figure 1. CT-Scan test for the bones.

The DICOM images are exported to MIMICS program to construct the three dimensional model. Program named 3-matic was used to generate the surface meshing. The quality of the meshing was tested by using height to base ratio. The volumetric meshing was generated by using elements type TET 4 (3-matic program has two option for the volumetric meshing TET 4 and TET 8). The meshed modal is exported to MIMICS program again for the material assignment. Based on the grayscale of each pixel in the region of interest the apparent density was calculated. The finite element modal is exported to ANSYS mechanical APDL for the modal analysis (Figure 2). Modal analysis is used for the calculation of the natural frequency for different modes of vibration. Figure 3 shows the process of construction the three dimensional finite element model.

Figure 2. FEA in ANSYS.

3-D Model and FEA

Figure 3. Three dimensional modal and FEA model.
Material and Experimental

The samples were collected from different cows (10 samples). Most soft tissue was removed carefully. The samples were stored in -20°C. Sulfuric acid is a very corrosive agent and to reduce its effect on the bone it was diluted (20-30% concentration and pH 1). The bone was submerged in acidosis medium for 24 hrs. The bone is taken out from the acidosis medium and washed with water. A white layer of Calcium sulfate was removed from the surface of the bone. The bone was left to dry.

Modal test was used to extract the natural frequency experimentally. The Modal test was conducted before and after acidosis treatment. The equipments used in the modal test are listed in the Table 1 below.

<table>
<thead>
<tr>
<th>No</th>
<th>Device</th>
<th>Company</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Impulse hammer type IH-1</td>
<td>YMC PIEZOTRONICS, INC</td>
<td>Sensitivity 25 mV/N</td>
</tr>
<tr>
<td>2</td>
<td>Dynamic signal Analyzer</td>
<td>YMC PIEZOTRONICS, INC</td>
<td>YMC 9004 IEPE</td>
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<tr>
<td>3</td>
<td>Miniature Accelerometer</td>
<td>YMC PIEZOTRONICS, INC</td>
<td>Sensitivity 100 mV/g</td>
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</table>

The modal test was done with free-free boundary conditions [9,10]. The free-free boundary condition was simulated by placing the bone on soft sponge. The bone was excited by hammering on different locations on the bone by using the impulse hammer. The load cell on the tip of the hammer record the striking force signal. The response is captured by using miniature accelerometer. The dynamic signal analyzer will plot the frequency response function (FRF) by using the two signals. Peaks were identified on the magnitude curve of the FRF. The natural frequency is extracted by the help of the phase angle diagram. The quality of the test was monitored by the coherence. Any FRF plot which has coherence lower than 1 is ignored [11]. Due to the free-free boundary condition the second strike was a challenging issue. A care must be applied when hitting the bone. Figure 4 shows the experiment setup.

RESULTS

The natural frequency for the bones before and after submerged in diluted sulfuric acid was extracted by using FEA and experimentally by impulse test. Five modes of vibrations were verified and extracted for each sample. Peaks were identified on the magnitude graph of the frequency response function. The natural frequencies were extracted by the help of the phase angle diagram. The extraction of the lower modes of vibration (natural frequency) is hard to be found and in many times we could not extract them. This happened because the soft rubber hammer tip did not provide enough energy to excite the lower modes. There were good agreements between the finite element and experimental results. The maximum error between the FEA and the experimental was less than 10%. Table 2 below shows the natural frequency the bones before and after the treatment. Several frequencies were extracted for different modes of vibration.

<table>
<thead>
<tr>
<th>NO</th>
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<th>Fresh</th>
<th>Acidosis Treatment</th>
<th>Percentage Increase (%)</th>
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<tr>
<td>1</td>
<td>A1</td>
<td>Exp</td>
<td>Exp</td>
<td>FEA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5358</td>
<td>5541</td>
<td>5349</td>
</tr>
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<tr>
<td></td>
<td></td>
<td>7666</td>
<td>8056</td>
<td>9018</td>
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</tbody>
</table>

Figure 4. The modal test experiment setup.
**DISCUSSION**

The bone mechanical properties has strong correlation to the bone mineral density. Disease or calcium regulation has strong impact on the bone density which will change the mechanical properties of the bone. The natural frequency depends on the mechanical and geometrical properties of the bone. Therefore, natural frequency changes with the changes affecting the bone. The later can used as indication about the health condition of the bone. As we stated before the acidosis medium inside the body can found due to the extensive exercise and due to the presence of the metabolic acids. The body will try to neutralize those acids by using the calcium from the bones (the bones are the reservoir of calcium for the body). This procedure will reduce the calcium content inside the bone. Eventually the bone density will be reduced.

There were good agreement between the FEA and experimental; results (less than 10%). The variation between the two results is due to the free-free boundary condition simulation because we can’t achieve perfectly the free-free boundary condition experimentally.

The results shows that there is increase in the natural frequency after the treatment. This due to the mss lost in the chemical reaction (Calcium sulfate). The percentage of increase in the natural frequency vary from 2-9%. This variation can be related to variation in the sulfuric acid concentration (20-30%).
CONCLUSION

The Acidic medium simulate the effect of calcium efflux from the bone. Studies have proved that there is a link between the pH factor and the osteoporosis disease. This study give us better understanding about the effect of the acidic medium on the bone natural frequency. The results shows that the natural frequency of the bone is increased due to the loose of mss (chemical reaction). Although, the effect of bone disease does not affect the inorganic materials inside the bone matrix but we need to have better understanding about the influence of calcium efflux on the natural frequency of the bone. The aim of all the studies which are dealing with frequency analysis of the bone is to develop nonradioactive test to measure the bone health condition without exposing the patient to radiation hazard. This aim require the understanding the influence of each element inside the bone matrix on the natural frequency.

CONFLICT OF INTEREST

No outside funding or grants in support of our research for or preparation of the work is received. And no personal or institutional financial support is related to the study. Furthermore, we have had full control of all primary data and we agree to allow the journal to review the data if recommended. Therefore, we accompany this submission with Authorship Responsibility and Disclosure Regarding Commercial Interest form.

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REFERENCES