INTRODUCTION Bone has a highly hierarchical structure that determines their mechanical behavior at multiple (i.e., microstructural, ultrastructural, and molecular) length scales [1]. The bulk mechanical behavior of bone tissues has been well documented [2]. However, the relationship between the bulk behavior and the ultrastructure of bone is still poorly understood. It is extremely difficult for conventional methodologies to measure the tissue properties at such small length scales. Recent progress in nanoindentation technology allows for measurements of elastic modulus and hardness of bone tissues [3]. Unfortunately, however, the nanoindentation techniques are not suitable for assessing the post-yield to failure behavior of bone at ultrastructural levels, which is critical to understanding the failure mechanism of the tissue.

Among the currently available nano techniques, simple scratch tests may lead to removal of surface material, thus allowing for estimation of the capability of the material to dissipate energy till failure (or toughness). So, scratching tests seem to be a potential candidate to measure the failure energy dissipation or toughness of bone. However, so far scratch tests were used only for assessing the resistance of materials to wear, friction of paired surfaces, thin film and coating properties, or studying machining properties of engineering materials (metals, ceramics, and polymers). Since bone is a quasi-brittle material with an appreciable but limited plastic deformation, the energy dissipation by tissue removal during a nanoscratch test may be used to evaluate the in situ toughness of bone tissues [4]. The objective of this study was to validate the efficacy of the test methodology of nanoscratch using bones from osteogenesis imperfecta mouse (oim) models, which are known to have various degrees of brittleness [5, 6].

METHODS Nanoscratch is the combination of an indentation process and a cutting action, which may result in removal of material due to deformation and failure in front of the scratch tip. Based on the mechanics of cutting process, the material in front of the scratch tip is forced to flow mainly along a shear plane (Figure 1). As the tip proceeds with certain distance, a part of volume of the material in front of the tip would be removed along the plane by either plastic flow or failure. Since bone is a quasi-brittle material with an appreciable plastic deformation, a limited plastic flow along the shear plane would occur in the early deformation, and then followed by the failure and removal of the tissue along the plane. The energy dissipated from the removal of bone tissues by the cutting action can be directly used as a measure of the in situ toughness of the tissue (i.e., the energy dissipation until failure).

Femurs from three groups of male mice (oim/oim, oim/+ and +/+ N=6 per group) were used in this study. The oim/oim mouse has a severe type of osteogenesis imperfecta (OI), the oim/+ mouse has a mild type of OI, and the +/+ mouse is the wild-type control group. Mouse femurs were harvested after all mice were sacrificed at the age of 5 months old. The femurs were then embedded in plastic resins and the cross section of mid-diaphysis of the specimens was sliced, polished, and mounted on a custom designed specimen holder.

Nanoscratch tests of mouse femurs were performed on an MTS nanoindenter XP system using a cube corner diamond tip with a scratch length of 20µm and a normal load of 5mN. During the scratching, penetration depth and frictional force along the scratch was measured (Figure 2). At the end of the profile of the nanoscratch test, the residual depth and scratch width were measured (Figure 2).

One-way analysis of variance (ANOVA) was performed to answer the question whether the in situ toughness measured by nanoscratch tests of femoral bones was significantly different among severe-type, mild-type and wild-type mouse models. Furthermore, a post-hoc analysis using the turkey test was conducted to compare toughness values between mouse groups. The significant level of these statistical tests was set as p-values less than 0.05.

RESULTS: Analysis of variance indicated significant differences of in situ toughness (p<0.001) among femurs from severe-type, mild-type and wild-type mice (Table 1). From the post-hoc analysis, significant differences were detected between groups. The severe-type mouse femur had the lowest toughness (2.19±0.41 GJ/m²) whereas the wild-type had the highest value (3.57±0.22 GJ/m²). The value of in situ toughness for the mild-type mouse femur (2.97±0.37 GJ/m²) was in-between.

In addition to toughness, ANOVA also showed significant differences of scratch width, penetration depth, residual depth and frictional force for three mouse groups (Table 1). It was worth noting that the residual depth and scratch width of the severe-type femur were the highest whereas the frictional force was the lowest. The largest volume of removal and the smallest energy required during the nanoscratch test contributed to the lowest toughness value for the severe-type mouse femur.

Table 1 Comparison of parameters from nanoscratch tests for three groups of oim mouse models

<table>
<thead>
<tr>
<th>Group</th>
<th>Scratch Width (µm)</th>
<th>Penetration Depth (nm)</th>
<th>Residual Depth (µm)</th>
<th>Frictional Force (mN)</th>
<th>Toughness (GJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>4627±319</td>
<td>1402±56</td>
<td>905±41</td>
<td>7.4±0.35</td>
<td>3.57±0.22</td>
</tr>
<tr>
<td>Mild-type</td>
<td>5000±364</td>
<td>1335±55</td>
<td>903±33</td>
<td>6.9±0.83</td>
<td>2.97±0.37</td>
</tr>
<tr>
<td>Severe-type</td>
<td>5457±571</td>
<td>1474±117</td>
<td>1083±115</td>
<td>6.3±0.27</td>
<td>2.19±0.41</td>
</tr>
</tbody>
</table>

ANOVA: p=0.02

DISCUSSION: Conventional mechanical testing such as four-point bending and torsion has indicated reduction of mechanical properties in femoral bones of severe-type (oim/oim) and mild-type (oim/+), compared with the wild-type (+/+) [5, 6]. In particular, the energy-to-failure of femoral bones from four-point bending tests was the lowest in the severe-type, the highest in the wild-type, and intermediate in the mild-type. The results from these studies are in good agreement with in situ measurement of toughness values by nanoscratch tests. Therefore, the efficacy of nanoscratch tests has been validated in this study. Consequently, nanoscratch tests may open a new avenue for researchers to explore the ultrastructural properties of bone and other hard tissues and to elucidate the underlying mechanisms of traumatic and pathologic skeletal fractures.

ACKNOWLEDGEMENTS: This study was supported by a grant from NSF (Award#0900753). The authors would like to thank Mr. Siyuan Ding for preparing bone samples for nanoscratch tests.