EFFECT OF FATIGUE ON THE BONE MINERAL

S.P. Kotha and N. Guzelsu

1 Biomedical Engineering, Rutgers University, Piscataway, NJ 08854
2 Biomechanics, Tr# 4, 675 Hoes Lane-UMDNJ, Piscataway, NJ 08854
Email: guzelsu@umdnj.edu

INTRODUCTION

At the ultrastructural level, debonding at the mineral organic interface was postulated to be the cause of damage in osteons (Ascenzi et al., 1998). It was shown that the bone mineral crystallites were more disoriented while the collagen fibrils were not affected by cyclic loading in which the final loading was the compression cycle. The objective of this study is to investigate if fatigue testing in tension of cortical bone samples would lead to a greater orientation of the bone mineral crystallites in the direction of loading and if the interplanar spacing between the (002) planes of the bone mineral would change.

METHODS

Bovine plexiform femoral bone from 12 to 18 month old steer were obtained from a local slaughterhouse (MOPAC, Souderton, PA). The lateral and medial quadrants were machined into dumbbell shaped mechanical test specimens (Kotha et al., 1998). Some specimens were loaded in tensile fatigue for 8 cycles in order to damage them and the remaining were taken as control samples. The load-deformation curves that were recorded indicated that the fatigued bone samples were loaded past their yield point. Control and fatigued bone specimens were cut into five 2mm thick sections perpendicular to the length of the test specimen. After the samples were cut, the samples were left to dry in air for a day. X-Ray diffraction (XRD) was performed on these sections within four days of mechanical testing. The cut pieces were placed with their lengths perpendicular to the plane of the holder. Since most of the (002) planes of the bone mineral are oriented perpendicular to the long axis of bone, the (002) planes of the bone mineral are parallel to the plane of the holder. XRD was performed on a Philips Analytical X-Ray diffractometer (PW3050-MPD) using Ni-filtered Cu Kα radiation, divergent slit of 1° and a receiving slit of 0.05°, operating voltage of 40 kV and a current of 45 mA. The samples were scanned at a stepwidth of 0.02° two theta (2θ), with a measuring time of 10 seconds over a scan range from 23 to 28 degrees 2θ. Measurements of the background were taken for later subtraction. The scans were background corrected and were Kα2 stripped to improve angular accuracy. No smoothing of the XRD profile was done. The position of the (002) peak and the full width at half maximum (FWHM) of the scans was obtained using the software provided with the diffractometer. The FWHM of the (002) peak was calculated by the equation:

\[ \beta_{002} = (\beta_{\text{sample}}^2 - \beta_{\text{standard}}^2)^{1/2} \]

where \( \beta_{\text{standard}} \) is the instrumental broadening obtained from the powdered standard (SiO2). The values for the unit cell parameters were calculated using Bragg’s law: \( \lambda = 2d \sin \theta \), where ‘\( \lambda \)’ is the wavelength of the incident beam, ‘\( \theta \)’ is the bragg angle and ‘\( d \)’ is the value of the spacing between the (002) planes.

RESULTS
The FWHM of the 002 peak of fatigued bone is smaller compared to that of control bone (Table 1). The positions of the 002 peak are similar in both fatigued and control bone (Table 1). The interplanar spacing of the (002) planes is shown not to change with fatigue loading.

DISCUSSION
The results show that the FWHM of the 002 peak decreased due to fatigue testing (Table 1). This indicates that the bone mineral orient towards the direction of applied load. Since the position of the 002 peak of the bone mineral did not change due to fatigue loading, there is little change in crystallite strain due to fatigue loading. Therefore, the interplanar spacing between the (002) planes of the bone mineral does not change in the fatigued and control bones. The decrease in the FWHM of the 002 peak indicates that the increased number of bone mineral crystallites contributing to the diffraction process is more than the decrease in crystallite size along the c-axis of the bone mineral crystallite. This would indicate that few of the bone mineral crystallites break during fatigue loading. The presence of the organic in bone decreases the intensity of the diffracted x-rays. The intensity obtained by using a powder standard (SiO₂) is similar to the intensities obtained from the 002 peak of the bone mineral in intact bone pieces. This indicates that the broadening in the broadening in the 002 peak of the bone specimens (βstandard). Bone proteoglycan has been shown to change orientation by 5° while the collagen does not, due to compressive or torsional loading (Skerry et al., 1990). Assuming the bone proteoglycan to be associated with some of the bone mineral crystals, some of the bone mineral would change orientation due to tensile loading. Though the mode of loading as well as the ultrastructure of the bone used was different, we assume the results to apply to this study. This would indicate that some of the bone mineral crystals along with the associated non-collagenous bone matrix reorients between 3°- 4° towards the direction of applied load while the collagen fibrils do not change orientation. This leads to some bonding changes between the mineral with its associated non-collagenous organic and the remaining collagenous organic. It is shown that there is no change in the interplanar spacing of the (002) planes.

REFERENCES

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Table 1: X-Ray Diffraction measurements (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Half-Width at Half Max (2θ°)</th>
<th>Peak Location (2θ°)</th>
<th>Interplanar Spacing A°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.267 ± 0.010ᵃ</td>
<td>25.89 ± 0.03ᵃ</td>
<td>6.699 ± 0.007ᵃ</td>
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<tr>
<td>Damaged</td>
<td>0.220 ± 0.008ᵇ</td>
<td>25.89 ± 0.02ᵃ</td>
<td>6.698 ± 0.005ᵃ</td>
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