Osteon Pullout in the Equine Third Metacarpal Bone: Effects of Ex Vivo Fatigue

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An important concept in bone mechanics is that osteons influence mechanical properties in several ways, including contributing to toughness and fatigue strength by debonding from the interstitial matrix so as to "bridge" developing cracks. Observations of "pulled out" osteons on fracture surfaces are thought to be indicative of such behavior. We tested the hypothesis that osteon pullout varies with mode of loading (fatigue vs. monotonic), cortical region, elastic modulus, and fatigue life. Mid-diaphseal beams from the dorsal, medial, and lateral regions of the equine third metacarpal bone were fractured in four point bending by monotonic loading to failure under deflection control, with or without 105 cycles of previous fatigue loading producing 5000 microstrain (15-20% of the expected failure strain) on the first cycle; or sinusoidal fatigue loading to failure, under load or deflection control, with the initial cycle producing 10,000 microstrain (30-40% of the expected failure strain). Using scanning electron microscopy, percent fracture surface area exhibiting osteon pullout (%OP.Ar) was measured. Monotonically loaded specimens and the compression side of fatigue fracture surfaces exhibited no osteon pullout. In load-controlled fatigue, pullout was present on the tension side of fracture surfaces, was regionally dependent (occurring to a greater amount dorsally), and was correlated negatively with elastic modulus and positively with fatigue life. Regional variation in %OP.Ar was also significant for the pooled (load and deflection controlled) fatigue specimens. %OP.Ar was nearly significantly greater in deflection controlled fatigue specimens than in load-controlled specimens (p=0.059). The data suggest that tensile fatigue loading of cortical bone eventually introduces damage that results in osteonal debonding and pullout, which is also associated with increased fatigue life via mechanisms that are not yet clear.

Introduction

Osteonal debonding and pullout have been identified as mechanisms for toughening cortical bone by reducing strain energy and controlling crack propagation [21]. Pullout is thought to occur when the tensile strength of an osteon exceeds the shear strength at its cement line or interlamellar interfaces, causing the osteon to debond and bridge a propagating crack, and then be pulled out of the fracture surface in a telescoping fashion (Fig. 1). This phenomenon has been observed in cortical specimens from various species fractured in tension or bending at slow strain rates [1,3,5,6,16,17,20,21,23,24].
This paper describes variations in osteon pullout behavior within the dorsal, medial, and lateral regions of the cortex of the equine third metacarpal bone under various loading conditions. Our previous experiments on machined specimens from this bone demonstrated that monotonic strength, elastic modulus, and flexural fatigue life vary across these regions [9,13,15]. Specifically, the lateral region is stiffer and monotonically stronger than the dorsal region, but the latter has greater fatigue life [9]. We also found regional variations in histomorphometric measures of osteon remodeling and structure [12,14]. In the lateral region, remodeling declines with age and longitudinal collagen fibers pre-dominate; conversely, the remodeling rate is maintained and collagen fibers are more transversely oriented in the dorsal region. Furthermore, the lateral region has larger osteons and less porosity than the dorsal region. These observations suggested that average osteonal age (and by implication, mineralization and stiffness) and structure are regionally dependent, and this raised the question of whether osteon pullout would exhibit similar regional variations that could help explain the observed differences in fatigue life and other mechanical properties.

Although osteon pullout has been observed in various species, we have found no reports for any species describing either intracortical regional variations in this phenomenon or how it is related to modulus, fatigue life, or fatigue loading conditions. Therefore, we tested the hypothesis that the amount of pullout occurring during fracture of beams made from equine third metacarpal cortical specimens would vary with mode of loading (fatigue vs. monotonic), cortical region (dorsal, medial, lateral), elastic modulus, and fatigue life.

**Methods**

The investigation utilized archived specimens from three different experiments (Table 1) [8,9]. Each experiment tested pairs of third metacarpal bone specimens from six different Thoroughbred race-horses. In each experiment, three rectangular beams (4 x 10 x 100 mm) were machined from each bone, one each from the dorsal, lateral, and medial regions of the diaphysis, with the beam’s long axis parallel to that of the bone. All three experiments had been designed so that the six individual horses, three cortical regions, and left/right sides (except as noted below) were equally represented among the experimental groups. All 36 beams in each experiment (six horses x two bones x three regions) had been loaded to failure in symmetric 4-point bending, either monotonically under deflection control at a rate of 1 mm/s or by sinusoidal fatigue loading at 2 Hz using load or deflection control. In all cases, the inner and outer supports were 32 and 64 mm
apart, respectively. Except as noted below, the periosteal side of the beam received tensile stresses.

Experiment 1: Monotonic failure with or without prior fatigue

There were two experimental groups. The first group (n=12) was fatigued for 100,000 cycles under load control at loads calculated to produce an initial strain range of 0-5000 microstrain (mean initial strain rate=0.020 s⁻¹), then monotonically loaded to failure. The fatigue loading produced strains on the order of 15-20% of the failure strains for similar specimens [9]. The second group (n=12) was loaded monotonically to failure (strain rate=0.0043 s⁻¹) without any prior fatigue loading. (The remaining 12 beams were not used in the experiments reported here. Note also that for consistency with Experiments 2 and 3, we analyzed specimens from a previously unpublished 4-point bending residual strength experiment rather than those from a similar experiment [13] that utilized 3-point bending.)

Experiment 2: Monotonic vs. load control fatigue failure

There were two experimental groups. The first group (n=12) was monotonically loaded to failure at a strain rate of 0.0043 s⁻¹. The remaining beams (n=24) were fatigue loaded to failure under load control at loads calculated to produce an initial strain range of 10⁴ microstrain. These fatigue loads produced strains that were on the order of 30-40% of the failure strains for similar specimens [9]. The initial mean strain rate was 0.040 s⁻¹ this increased as the elastic modulus degraded during the failure process. Half of these 24 beams were loaded with the periosteal side in tension, and half with the endosteal side in tension. However, this had not affected the fatigue life or other mechanical properties [9], so they were treated as a single group in the present experiment.

Experiment 3: Load vs. deflection control fatigue failure

In this experiment, beams machined from left and right bones were placed in load and deflection control fatigue groups, respectively (Previous work had shown no left-right differences in any mechanical or histomorphometric properties [9,15]). The load control group (n=18) was fatigued as described in Experiment 2. The deflection control group (n=18) was cycled between zero load and the deflection originally associated with 10⁴ microstrain, as determined by
beam theory, until failure. The mean strain rate was 0.040 s⁻¹ for each cycle throughout the deflection control tests.

Fracture surfaces were typically characterized by a transverse failure plane perpendicular to the beam’s long axis on the tensile side and an oblique failure plane approximately 45° to the beam axis on the compressive side (Fig. 2). (Sometimes a second oblique fracture plane on the compressed side of the complementary fracture fragment would create a third, "butterfly" fracture fragment, but these were not studied.) One of the fragments containing an entire cross-section of the beam was arbitrarily chosen for analysis. To remove soft tissues, each specimen was sequentially immersed for 24 h in a 70% ethanol solution, 7.5% Clorox solution, and acetone. Specimens were then sonicated and rinsed in acetone four-five times before being air dried in a fume hood and further dried in a vacuum oven at 60°C for 24 h. Dried specimens were sputter coated with gold to a thickness of 300 Å (Model ES 100 Sputter Coater; Polaron, Fisons Instruments, Mountain View, CA) and observed in a scanning electron microscope (ISI-DS-130 or ISI E5200; Topcon Technologies, Pleasanton, CA) at 10 kV. For each of the three experiments, specimens were randomized and blinded for analysis. Experiments 1 and 3 were analyzed by LPH, and Experiment 2 was analyzed by SMS.

The fracture surface of each specimen was photographed parallel to the beam’s longitudinal axis at a magnification allowing a perspective of the entire surface (~10x magnification). Two additional photographs at 20x magnification were then taken for measurement of the amount of the fracture surface exhibiting osteon pullout. These "measurement images", slightly overlapped one another and had a resolution of approximately 4500 pixels/mm². They were analyzed using NIH Image software (Scion Image for Windows). The total area of the fractured surface (Fx.Ar) was obtained by tracing its boundaries in the two measurement images. The osteon pullout area (OP.Ar) was obtained by tracing the portion(s) of this area exhibiting the roughened surface texture characteristic of this phenomenon, with incomplete or complete extrusion of debonded osteons from the surrounding matrix or cavities in the fracture surface from which osteons had been "pulled out." Percent osteon pullout area was then calculated for each specimen as %OP.Ar = 100 x OP.Ar/Fx.Ar.

Additional photographs at 100x and 400x magnification were taken and used at times to confirm the presence of osteon pullout. Anatomic landmarks were used to avoid duplicate measurement of overlapped surface areas. It was recognized that portions of the fracture surface were oriented obliquely to the focal plane and would thus present as reduced surface areas.
However, since Fx.Ar and OP.Ar would be equally reduced, there was no need to adjust the computation of %OP.Ar for this effect.

SAS statistical software (Version 6.12; SAS Institute, Cary, NC) was used for data analysis with p < 0.05 the criterion for statistical significance. Each experiment was analyzed separately because they were done at different times and because each experiment was designed to control for the individual differences between its horses. The effects of mode of loading (load control, deflection control) and region (dorsal, lateral, medial) on %OP.Ar were assessed with repeated measures or mixed model analysis of variance that treated horse as a random effect, assumed there was no side effect (left vs. right) [9.15], and included all possible two-way interactions. Post hoc contrasts were performed between the levels of region. Comparable non-parametric tests were also used when a substantial number of specimens had % OP.Ar=0. These tests included chi-square, Fisher’s exact test (one effect assessed at a time), and Friedman statistics (assessing one variable while controlling for horse). Finally, simple linear regressions were utilized to assess the relationships between %OP.Ar and elastic modulus or the logarithm of the number of cycles to failure (log Nf).

Results

The specimens in all experiments failed by complete fracture, but with substantial variations in morphology (Figs. 3 and 4). Some portions of the transverse fracture surfaces exhibited markedly irregular topography with peaks and valleys caused by segments of osteons that had pulled out of the opposed matrix. Other portions of these transverse fracture surfaces lacked such evidence of osteon pullout, being distinctly smoother with peaks and valleys of much smaller magnitude. The incidence and amount of osteon pullout appeared to increase with distance from the neutral axis, presumed to be near the junction of the transverse and oblique fracture surfaces.

The oblique fracture surfaces, commonly associated with shear failure, were much smoother than the transverse fracture surfaces (Fig. 2). The surface seemed to follow the lamellar interfaces in a stepwise fashion for variable distances, such that longitudinal sections of Haversian canals could be observed. However, under higher magnification, no osteon pullout was discernible on these oblique fracture surfaces.

In Experiment 1 (monotonic failure with or without prior fatigue), the only specimen exhibiting osteon pullout had failed to complete the fatigue protocol, fracturing at 58,998 cycles. It exhibited osteon pullout over 15.2% of its fracture surface area (Table I). Consequently, osteon pullout was entirely absent under monotonic failure conditions, regardless of cortical region or whether
the specimens had been subjected to prior fatigue loading or not.

In Experiment 2 (monotonic vs. load control fatigue failure) one lateral and one dorsal specimen were un-available for examination. There was again no discern-able amount of osteon pullout in the monotonically failed specimens. In those beams that were failed in fatigue, osteon pullout was absent in all seven lateral region specimens, but did occur in six of seven dorsal specimens and seven of eight medial specimens. %OP.Ar in the dorsal region was 12.6 ± 4.1%, which was statistically different from the other two regions; %OP.Ar in the medial region (5.9 ± 1.7%) was not significantly different from that in the dorsal or lateral regions (Table 1). Osteon pullout was highly correlated with elastic modulus and logNf (both p < 0.0001). Variations in elastic modulus accounted for 43% of the variability in %OP.Ar (Fig. 5A), while 66% of the variability in %OP.Ar was attributable to variations in logNf (Fig. SB).

In Experiment 3 (load vs. deflection control fatigue failure) osteon pullout was observed under both control modes: 9 of 18 load control specimens and 12 of 18 deflection control specimens (Table 1). When repeated measures analysis of variance was used to test for the effects of deflection vs. load control and cortical region on the amount of osteon pullout, deflection control was found to result in nearly significantly more osteon pullout than load control (p = 0.059), but neither region (p = 0.23) nor the region vs. loading mode interaction (p = 0.58) approached significance. When the load control and deflection control specimens were considered separately, the effect of region on %OP.Ar was not statistically significant for the deflection control specimens (p = 0.90); however, a statistically significant regional effect was found for load control specimens (p = 0.0072). %OP.Ar values for the lateral and medial regions were smaller than for the dorsal region (p = 0.0208 and p = 0.0427, respectively, Table 1), but not statistically different from one another (p = 0.11).

The findings regarding regional differences within each control mode were similar when the data were analyzed with non-parametric chi-square or Fisher exact tests, or with Friedman statistics (controlling for horse). However, when the deflection and load control groups were pooled, these non-parametric ranked tests revealed a significant regional difference, with greater %OP.Ar in the dorsal region: chi-square, p = 0.01; Fisher exact test, p = 0.02; Friedman statistic: p = 0.02 when controlling for horse or p = 0.03 when controlling for horse and loading mode.

Osteon pullout was significantly correlated with elastic modulus and logNf for load
control specimens (p < 0.0001 and p=0.03, respectively), but not for deflection control specimens (p=0.27 for both). Varia-
tions in elastic modulus and logNf accounted for 58% and 26%, respectively, of the variability in %OP.Ar for load control specimens (Fig. 5C and D), but these variables did not have a significant effect on %OP.Ar for deflection control specimens (Fig. 5E and F).

**Discussion**

We hypothesized that the amount of osteon pullout occurring during fracture would vary with the mode of failure (fatigue vs. monotonic), cortical region, elastic modulus, and fatigue life. The results supported each of these hypotheses. Osteon pullout was exclusively asso-
ciated with fatigue loading and absent in monotonic failure, significantly greater in the dorsal region than in the lateral region (at least in load control fatigue), negatively correlated with modulus for load control specimens, and positively correlated with fatigue life under load control. Thus, these results strongly support a fundamental concept in bone mechanics: that osteonal structure affects properties such as modulus and fatigue resistance in ways that may vary from site-to-site in the cortices of long bones.

This study had several limitations. First, identifying and circumscribing osteon pullout areas was subject to human judgment. Second, the applied strain range in the fatigue failure experiments was superphysiologic in order to produce fatigue failure in a reasonable amount of time at a physiologic loading frequency [15,18]. Also, the loading did not produce the same distribution of strain in the specimen as in vivo loading. Several in vivo studies have demonstrated axial compression in the third metacarpal bones of running horses, but the amount and direction of superimposed bending remain unclear [2,7,10,18]. In addition, every specimen’s orien-
tation had been marked by beveling one edge on the periosteal side prior to testing. %OP.Ar could have been affected by this practice because osteon pullout consis-tently occurred on the tensile side of the beam, and this was usually the periosteal side as well.

Six of the 18 deflection control specimens (one me-dial, five lateral) did not fracture during fatigue testing. These tests were terminated when the load range had diminished to 10% of the initial load range, and fracture was completed by monotonic loading. These specimens subsequently exhibited macroscopic cracks on their machined surfaces,
transverse to their long axes and predominantly on the compressive side. The use of monotonic loading to produce final fracture could have influenced the amount of osteon pullout that was observed in these specimens. However, since monotonic loading did not produce pullout in the other experiments, it seems unlikely that it would have contributed to the greater %OP.Ar in the deflection control specimens relative to those fatigued in load control.

Comparisons between the three experiments were impeded by methodological differences. Experiment 2 was analyzed by a different observer than Experiments 1 and 3, and the horses used for Experiment 1 were significantly younger than those used for Experiment 3: 2.8±0.7 vs. 4.0±0.6 years old. Previous work has shown that beyond 1-2 years of age, the remodeling rate in equine third metacarpal bones declines, and the cortex exhibits fewer resorption spaces and a higher degree of mineralization [19,25].

In spite of these limitations, the results may provide significant insight regarding damage and failure mechanisms in cortical bone. In theory, osteonal debonding and pullout occur as a result of shear failure at or near the cement line [22]. This may occur because osteonal cement lines are more viscoelastic than the adjacent bone due to their altered mineral content and protein composition [4,11]. A viscoelastic mechanism for pullout is consistent with observations that osteonal debonding during monotonic failure is more pronounced when loading rates and specimen geometries result in exceptionally slow crack propagation [3,21]. At higher strain rates, the cement line’s tendency to shear may substantially diminish, reducing pullout. In the current study, however, loading mode was found to be another, and potentially more important, determinant of osteon pull-out. Our fatigue and monotonic failure tests were conducted at different strain rates, with pullout observed at the higher strain rate: in fatigue failure at 0.040 s⁻¹ rather than in monotonic failure at 0.0043 s⁻¹. Perhaps during prolonged fatigue loading there is opportunity for cement line interfaces to accumulate shear damage prior to or during the initiation and propagation of a transverse crack like that depicted in Fig. 1. The fact that pullout did not occur in Experiment 1 specimens loaded to 5000 microstrain for 100,000 cycles (except for one specimen that failed during this preliminary fatigue loading) suggests two possibilities: (1) the osteonal debonding process occurs primarily in the later
stages of the fatigue process, after enough shear damage has accumulated, or (2) osteon pullout is sensitive to the strain range of cyclic loading. Alternatively, both of these mechanisms may be important.

%OP.Ar was nearly significantly greater in the deflection control specimens than in the load control specimens. This may have been related to the fact that during deflection control tests, the applied load declines as damage decreases the elastic modulus, and the energy input diminishes for each cycle. This in turn may contribute to longer fatigue lives than in load control tests, and allow more osteonal debonding to occur before fatigue failure.

Regional variation in osteon pullout was anticipated, as past research had demonstrated that the dorsal region possesses the longest fatigue life and the lateral region the shortest [8,9,15]. If osteon debonding is a major mechanism for energy dissipation and results in pullout at the time of fracture, the greatest amount of osteon pullout should occur in the dorsal region and the least in the lateral region. This was observed in the load control groups of Experiments 2 and 3, where 92% of dorsal specimens and 0% of lateral specimens exhibited pull-out. The deflection control group, by contrast, demonstrated osteon pullout in every region. Apparently, as tests progressed under deflection control, lateral region osteons experienced conditions more conducive to de-bonding and pullout than was the case under load control. This may be related to the fact that the applied strain energy diminishes with each cycle under deflection control, but increases under load control. Further studies of osteon mechanics in the dorsal and lateral regions may shed more light on this subject.

We have previously found that, in general, the lateral and dorsal regions have different osteonal as well as mechanical properties, with the medial region often having intermediate values. For example, the lateral region, which exhibited no pullout in load control fatigue, has osteons of larger diameter than the dorsal region (182±13 vs. 156±19μm) [12]. This is contrary to the theoretical suggestion that pullout is more likely when osteons are larger in diameter because this increases the osteon’s tensile strength relative to its peripheral (cement line) interfacial shear strength [22]. On the other hand, Moyle and Bowden [17] found that work of fracture in human bone (also associated with
osteon pullout in monotonic failure at very low strain rates) was minimal for osteons about 180 μm in diameter and increased for smaller (150-160 μm) as well as larger (210-220 μm) diameters. We previously found that mean osteonal diameter in the lateral region of the equine metacarpus was 182 ± 13 μm, compared with 156 ± 19 μm in the dorsal region [12]. Thus, our equine data are consistent with Moyle and Bowden's experimental results relative to osteon size. The discrepancy with the tensile vs. shear strength theory may result from other osteonal characteristics.

We have also studied collagen fiber orientation in the equine metacarpus using polarized light microscopy. Averaging collagen birefringence over beam cross-sections has shown that the lateral region's collagen is significantly more longitudinally oriented than that of the dorsal region [14]. In addition, we have identified a kind of osteonal architecture that we call "hooped osteons," in which the inner collagen fibers are longitudinally oriented, and the peripheral fibers adjacent to the cement line are circumferential [12]. This osteonal structure was significantly more common in the lateral than the dorsal region. If these "hoops" help bind the osteon to the surrounding bone, perhaps their relative absence contributes to osteon debonding in the dorsal region.

In summary, we have shown that the amount of osteon pullout associated with fracture of equine cortical bone depends on the mode of loading and cortical region. Within the range of conditions described here, pullout was present in fatigue but not monotonic bending failure, even when the latter followed substantial previous fatigue loading. In two separate experiments, we found that load control pullout was regionally dependent, appearing in the dorsal cortex where previous studies have shown that collagen fibers are more transversely oriented, osteons are smaller, and fatigue lives are longer [12]. Conversely, we found pullout was absent in the lateral region, where previous studies showed that collagen fibers are more longitudinally oriented, osteons are larger and more "hooped," and monotonic strength and stiffness are greater [12]. When similar experiments were done under deflection control pullout was equally present in all three cortical regions. It would be of great interest to understand more about the specific aspects of osteon structure that contribute to pullout, and how these features are controlled during the bone remodeling process.
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References


Fig. 1. Schematic diagram illustrating the postulated mechanism of osteon pullout. Tensile loads produce a crack that advances transversely across intact osteons embedded in the interstitial matrix. The matrix shears from the osteon cement line, resulting in debonding and osteonal crack bridging. Eventually the bridging osteons fail in tension. When they fail at sites below or above the crack surface, osteons form pits and projections, respectively, producing the irregular morphology characteristic of osteon pullout. Relative to that of the experimental specimens, the volume fraction of osteons is intentionally reduced here for clarity of illustration.

Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Experimental group</th>
<th>Dorsal</th>
<th>Spec. pullout ratio</th>
<th>Medial</th>
<th>Spec. pullout ratio</th>
<th>Lateral</th>
<th>Spec. pullout ratio</th>
<th>All regions</th>
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<tr>
<td></td>
<td></td>
<td>%OP.Ar Mean ± SD</td>
<td>Spec. pullout ratio</td>
<td>%OP.Ar Mean ± SD</td>
<td>Spec. pullout ratio</td>
<td>%OP.Ar Mean ± SD</td>
<td>Spec. pullout ratio</td>
<td>%OP.Ar Mean ± SD</td>
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<td>1. Monotonic failure with/without prior fatigue</td>
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<td>0/3</td>
<td>0 ± 0</td>
<td>0/4</td>
<td>0 ± 0</td>
<td>0/4</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Without prior fatigue</td>
<td>0 ± 0</td>
<td>0/4</td>
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<tr>
<td></td>
<td>Monotonic vs. load control fatigue failure</td>
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<td>6/7</td>
<td>5.9 ± 1.7b</td>
<td>7/8</td>
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<td>0/7</td>
<td>6.2 ± 8.3</td>
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<td>Fatigue failure</td>
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<td>6/6</td>
<td>6.5 ± 8.3b</td>
<td>3/6</td>
<td>0 ± 0b</td>
<td>0/6</td>
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<td>4/6</td>
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<td>5/6</td>
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<td>3/6</td>
<td>20.4 ± 18.2</td>
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Rows identify the two experimental groups in each of the three experiments. Columns show the experimental results for each anatomical region, the means for all regions, and the total number of beams successfully tested for each experimental group. Different alphabetical superscripts within a row indicate statistically significant differences (p < 0.05) in %OP.Ar. *Ignores one specimen that failed during fatigue preloading, prior to monotonic testing. This specimen's %OP.Ar was 15.2%. 
Fig. 2. Scanning electron micrograph (low power view at a 45° angle to the beam’s neutral plane) of a typical fracture surface from a cyclically loaded dorsal specimen. Inset diagram illustrates typical fracture fragment configuration (viewed from a point rotated about the longitudinal axis of the specimen in comparison to the micrograph) and the orientation of the neutral plane (---) at initiation of loading. The plateau (P) is on the tensile side of the specimen. The oblique portion of the fracture surface (O) is between this plateau and the narrow transverse shelf (S) adjacent to the compressive surface (C) of the specimen. A semi-circular region of osteon pullout (arrows) on the plateau has a rougher, textured appearance than the surrounding region of the transverse surface.
Fig. 3. Scanning electron micrograph of an osteon on the transverse fracture surface of a cyclically loaded dorsal specimen. Disruption of interlamellar and cement line interfaces reveals concentric lamellae and the outer osteon boundary of “pulled out” osteons.

Fig. 4. Scanning electron micrographs illustrating lack of osteon pullout on portions of the transverse fracture surfaces (top) and on all oblique fracture surfaces (bottom). Although a concentric ring pattern can be visualized around the centrally located Haversian canal, concentric lamellae are difficult to discern because of the flat fracture surface. Cyclically loaded dorsal specimen.
Fig. 5. Graphs depicting the relationships between osteon pullout and initial elastic modulus (left column) or fatigue life (right column); for load control specimens from Experiment 2 (A, B) and Experiment 3 (C, D), and for deflection control specimens from Experiment 3 (E, F). Note that $x = \log N_f$ in the right column’s regression equations.