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The Effect of Bone Microstructure on the Initiation and Growth of Microcracks[#]

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Abstract

Osteonal bone is often compared to a composite material and to metals as discontinuities within the material may provide sites of stress concentration for crack initiation and serve as barriers to crack growth. However, little experimental data exist to back up these hypotheses. Fluorescent chelating agents were applied at specific intervals to bone specimens fatigue tested in cyclic compression at a stress range of 80 MPa. The failed specimens were sectioned and labelled microcracks identified using UV epifluorescence microscopy. Microcrack lengths were measured and their relationship to cement lines surrounding secondary osteons recorded. Microcrack length at the time of encountering a cement line was also measured. Microcracks of less than 100 μm stopped growing when they encountered a cement line. Microcracks of greater than 100 μm in length continued to grow after encountering a cement line surrounding an osteon. Only microcracks greater than 300 μm in length were capable of penetrating osteons and these microcracks were the only ones which were observed to cause failure in the specimen. These experimental data support the hypothesis that secondary osteons act as barriers to crack propagation in compact bone. However, it shows that this microstructural barrier effect is dependent on the crack length at the time of encountering an osteon. For the vast majority of cracks, osteons act as barriers to growth but for the minority of cracks that are long enough and do break through the cement line, an osteon may actually act as a weakness in the bone and facilitate crack propagation.

Keywords: microcrack, propagation, accumulation, osteon, barrier

Introduction

Fatigue damage in bone occurs in the form of microcracks. These microcracks have been shown to act as a stimulus for bone remodelling [1-6]. The process of microcrack accumulation in bone may eventually lead to stress fractures. These fractures occur commonly in athletes and soldiers engaged in high intensity, repetitive activities such as marching or running and happen when microcracks accumulate at a rate that exceeds the capacity for bone repair [7]. Alternatively, the process of microcrack accumulation in bone may be one of the causes of fragility fractures, which can occur in ageing bone when damage accumulates at 'normal' rates but the bone's repair mechanism is deficient [8].

The resistance of any material to fatigue failure is a function of its resistance to either the initiation or propagation of cracks or both. Secondary osteonal bone has often been compared to composite materials and to metals whereby discontinuities within the material (e.g. fibres, laminae, voids) may provide stress concentration sites for crack initiation, but they also serve as barriers to crack growth which may slow down or even halt crack propagation. A number of authors have mentioned the possibility of a microstructural barrier effect existing in bone [9-12], whereby the critical stage in the fatigue process is not the initiation of cracks but their propagation beyond microstructural sizes. Numerous studies have looked at microcrack interaction with bone microstructure. Carter and Hayes [13] observed bone microdamage created by cyclic flexural loading and found that separation of cement lines and interlamellar cement bands, as well as tensile cracks in interstitial bone, played major roles in the failure of bone specimens. Other studies [14] showed that the microstructure of the bone constrained the growth directions of microcracks while Schaffler et al. [15]

showed that features of the bone matrix ultrastructure, such as the collagen fibre-bone mineral relationship, encourage the formation of numerous small cracks but minimise the formation of larger, detrimental cracks. Schaffler et al [8] also suggested the majority of microcracks in cortical bone are found in the interstitial matrix between osteons and Boyce et al. [16] showed experimentally that microcracks developed in the interstitial tissue regions but stopped at the osteonal boundaries.

Recent work in this laboratory has developed a technique which allows microcrack growth to be monitored during the course of a mechanical fatigue test by the application of a series of fluorescent chelating agents [17,18]. These fluorescent markers are site specific as they bind to calcium ions lining the crack walls. Each agent fluoresces a different colour under UV light and so individual agents can be distinguished when viewed using UV epifluorescence microscopy. Experimental work has been carried out using this technique to study microcrack accumulation at different intervals during fatigue testing of compact bone [19]. In that study, pre-stained specimens were fatigue tested in cyclic compression with up to three other chelating agents applied during testing to label microcracks formed at different time intervals. Microcracks were found to initiate in interstitial bone in the early part of a specimen's fatigue life. Further accumulation of microcracks was then suppressed until the period late in the specimen's life. Cracks were also described in terms of location: (a) osteonal, where they were located entirely within a secondary osteon or traversed a cement line surrounding a secondary osteon, or (b) interstitial: where they were located completely in interstitial bone between osteons or around cement lines (but not penetrating these cement lines). Over 80% of all cracks were interstitial and did not penetrate secondary osteons.

This paper is an extension of that study and looks specifically at the relationship between crack length and propagation. To understand this phenomenon, the study looks at the crack length at the time of encountering the cement line surrounding a secondary osteon and seeks to determine whether this influences the crack's ability to continue to grow. Rather than splitting cracks into two categories as before [19], cracks are divided into five distinct categories to look in detail at how osteons influence crack propagation in secondary compact bone. This tests the hypothesis that a microstructural barrier exists in bone whereby the critical stage in the fatigue process is not the initiation of cracks but their propagation beyond microstructural sizes.

Materials and Methods

Compact bone samples were removed from the mid-diaphyses of bovine tibiae and machined into typical waisted, test specimens of 7 mm circular cross section using an established protocol [20,21]. Compressive fatigue tests were carried out in an INSTRON 8501 servo-hydraulic testing machine used in load control to apply an axial force to the specimens, which were enclosed in a small plastic bath to which the dyes could be added and removed. All tests were carried out at room temperature, at a frequency of 3 Hz, with a stress range of 80 MPa and a ratio between minimum and maximum stress of 0.1. Specimens were kept wet during all stages of machining and testing.

Prior to testing, the machined specimens were placed in a single vial of 0.0005M alizarin under 50 mm Hg vacuum for 16 hours to label any microdamage which existed prior to testing. A pre-determined sequence of dyes [18] was used to label

microcracks formed during testing. Testing was carried out in xylenol orange for the first 10,000 cycles of testing. Testing was stopped, the xylenol orange removed, the bath was rinsed with distilled water and calcein green added. Testing was then continued until 50,000 cycles had elapsed and the calcein green was replaced with calcein blue. Failure was defined using established criteria; a 10% reduction in stiffness [20] which generally coincided with the appearance of a large crack. Following testing, the gauge length of the specimens was removed using a diamond saw (Struers Miniton, Frankfurt, Germany). Sections of 250 μm were cut, handground to between 100 and 150 μm and mounted under a glass coverslip. They were examined using epifluorescence microscopy, their cross sectional areas obtained and microcracks identified and measured using established criteria (Table 1).

Microcracks were divided into five distinct categories: (i) microcracks which were located in interstitial bone and did not encounter secondary osteons (ii) microcracks which initiated in interstitial bone but, when they encountered secondary osteons, stopped growing outright (iii) microcracks which initiated in interstitial bone and, when they encountered secondary osteons, continued to grow and were deflected around the cement line but did not propagate into the circumferential lamellae of the osteon (iv) microcracks which initiated in interstitial bone and, when they encountered secondary osteons, penetrated the cement line and propagated into the osteon and (v) microcracks which encountered secondary osteons at both tips of the crack and did not propagate at either end. The relationship between microcrack length and location using the five distinct categories was then analysed.

In all specimens, one observer identified microcracks and from a number of preliminary tests, it was estimated that the lower limit for reliable crack detection was a length of 30 μm and errors in measured length were in the range of ± 10 μm. Paired t-tests were performed to compare individual sets of data in order to determine statistical significance. One-way analysis of variance (ANOVA) and pairwise multiple comparison procedures (Dunn's Method) were used to compare groups of data. A probability value of 95% ($p<0.05$) was used to determine significance.

Results

Fig. 1 shows a graph of microcrack length at each of the different intervals during testing. Propagating microcracks (labelled with two agents indicating growth at two different stages during testing) were found to be longer than microcracks formed at individual periods during testing (281 μm; S.D. 119 μm). No significant difference in length was found in microcracks formed at individual periods during testing (0-10,000 cycles, 10,000-50,000 cycles, 50,000 cycles to failure). The mean crack length of cracks formed during these periods was found to be 170 μm (S.D. 56 μm). Pre-existing microcracks which had formed prior to testing, were short in comparison to the other types (56 μm; S.D. 50 μm) and were found close to the outer surfaces of the test specimens and did not propagate during testing.

Fig. 2 shows an example of a crack from each of the five categories described. Fig. 3 shows a graph of mean crack length at the time of encountering an osteon for each category. One-way analysis of variance (ANOVA) showed that that crack length at the time of encountering an osteon significantly affected its ability to propagate ($p<0.05$). The first category shows that cracks which did not encounter osteons grew

to an average length of 198 µm (S.D. 75 µm) before growth was halted. The second bar illustrates that cracks of mean length, 95 µm (S.D. 26 µm), when they encountered a cement line surrounding an osteon, stopped growing outright. However the third bar shows that cracks that propagated to an osteonal cement line but were deflected into it and around the osteon rather than penetrating it had a mean length 174 µm (S.D. 47 µm). These cracks were usually observed to stop growing soon after encountering the cement line. The fourth category shows cracks which did actually manage to penetrate osteons: these were significantly longer ($p<0.05$) than the other categories (313 µm S.D. 116 µm). The fifth category illustrates cracks which encountered osteons at both tips. This usually happened in areas of high osteon density. Cracks in this category stopped growing outright when they encountered cement lines and their average length of 106 µm (S.D. 37 µm) is also comparable with the average spacing of 100 µm found between osteons in these areas [23].

Discussion

It has frequently been hypothesized that secondary osteons in compact bone greatly influence crack propagation to critical sizes, which may ultimately cause failure. This research attempts to explain how failure occurs in compact bone and in particular to explain why most cracks are small, do not propagate and remain at sub-critical lengths while others may propagate and ultimately cause failure. To understand this phenomenon, the study looked specifically at the crack length at the time of encountering the cement line surrounding a secondary osteon and sought to determine whether this influences the crack's ability to continue to grow.

The results show that microcrack length at the time of osteon encounter is a critical factor in its ability to propagate (Fig. 3). Microcracks of lengths no greater than approximately 100 μm when they meet a cement line, will stop growing (Fig 2b). However cracks in the range 150-300 μm continued to grow after encountering cement lines surrounding secondary osteons. In general it was observed that these were unable to penetrate the cement line surrounding secondary osteons but were likely to be deflected around the cement line and often ceased growing soon afterwards (Fig 2c). Only microcracks above 300 μm in length when they encountered osteons were able to penetrate the cement line (Fig 2d). Interestingly however, although microcracks which did not encounter osteons (this usually occurred in regions of the bone with a low osteon density which in this study was found to be on the lateral side of the tibiae) grew to moderate lengths (198 μm; S.D. 75 μm), they did not grow to critical lengths even though cement lines were not present to act as barriers to growth. Future research placing an emphasis on looking at crack propagation and failure mechanisms in bone that contains very low osteon densities might be worthwhile to answer the question of why these cracks in areas of low osteon density never caused failure. This data suggests that another mechanism may exist which causes certain cracks to propagate to failure.

Fig. 1 shows the mean crack length data for different periods during testing . Pre-existing cracks were significantly smaller than the other categories and were found close to the surface of the specimen and did not propagate during testing. No significant difference in length was found between microcracks formed at different individual periods during testing. However, propagating microcracks which grew during at least two stages of the specimen's life, were found to be longer (281 μm)

than microcracks formed at individual periods during testing. This length is similar to that of microcracks which penetrated cement lines (Fig. 3) indicating that microcracks which were formed at least as early as the second stage of the specimen's life and then continued to propagate have a greater chance of breaking through osteons.

From a general observation of the specimens, failure always occurred with the propagation of one or two long cracks to critical lengths rather than the coalescence of numerous small microcracks. An interesting observation was that these cracks always appeared to penetrate a cement line at some stage on the path to failure. Although no quantitative data exist to back up this hypothesis, Fig. 4 shows a typical example of two large cracks that were involved in specimen failure. As these cracks grew to macrocrack level, they penetrated the cement lines of numerous osteons. This was a recurring theme when the fracture surfaces were studied, all failure surfaces tended to show splitting of osteons often at the Haversian canals in the centre. Failure tended to occur with the critical growth of cracks, which penetrated cement lines, rather than the growth of cracks which were found in regions of the bone with few secondary osteons. This suggests that in the event of a microcrack growing to a sufficient length which allowed it to penetrate a cement line and eventually reach as far as the Haversian canal in the centre of the osteon, its length and stress intensity were large enough to allow its progression through the osteon and onto the next Haversian system. Thus, once the crack was long enough to break through one Haversian system, it would not be stopped by encounters with either cement lines or Haversian canals and the scenario of cracks propagating from Haversian canal to Haversian canal is consistent with the experimental observations in this study.

This might explain why some authors have hypothesized that secondary osteons act as weaknesses in the bone and encourage crack propagation while others have claimed that they acted as barriers to further growth. In our earlier paper [19] we proposed that cement lines surrounding osteons act as barriers to the growth of small microcracks. The data presented in this study support this hypotheses, however we have now shown that if microcracks do grow to certain lengths (approximately 300 μm), they are long enough to allow penetration of secondary osteons. If these cracks continue to grow through the concentric lamellae inside an osteon and have a high enough stress intensity value to break through the Haversian canal, they have a clear pathway with no barriers to further growth and failure is a likely result. This still begs the question as to why cracks in regions of low osteon density were generally observed not to cause failure and suggests that perhaps cracks preferentially seek out osteons. Numerous studies have shown that secondary osteonal bone is mechanically weaker than primary bone [13, 24, 25] so failure will be more likely to occur in regions of secondary osteonal bone than in an adjacent region of primary bone. Although osteons act as barriers to growth, it is likely that they are actually weaker then the surrounding bone. Frasca [26] showed that the cement line interface between osteons and interstitial bone is relatively weak which means that it may reduce the shear strength of osteonal bone but slipping at this interface may relax shear stresses, reduce strain energy and therefore may slow crack propagation. Burr et al [27] showed that the cement line is a region of reduced mineralization and provides a relatively ductile interface with the surrounding bone matrix which has the qualities required to promote crack initiation but slow crack growth in compact bone. In fibre-reinforced composite materials, the crack can enter the fibre interface and then become trapped by deflection and blunting. If this is true for bone then it might imply

that the long cracks which have penetrated a cement line, are attracted to the stress concentration associated with Haversian canals and this increases the number that they encounter. This is worthy of further investigation.

In conclusion, this study further demonstrates the concept of a microstructural barrier effect existing and having a major effect on the fatigue behaviour of bone. It shows that for the vast majority of cracks, osteons act as barriers to growth but for the small number of long cracks that break through the cement line and split through a Haverisan canal allowing a pathway for further propagation and eventually failure, an osteon may actually act as a weakness in the bone and encourage further propagation.

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Fig. 1. Microcrack length at each of the different intervals during testing. Pre-existing microcracks were significantly shorter than the other categories. No significant difference was found in microcracks formed at individual periods during testing (0-10,000 cycles, 10,000-50,000 cycles, 50,000 cycles to failure) while propagating microcracks were found to be significantly longer than the other categories.

Fig. 2. An example of a crack from each of the five categories; (a) microcracks which were located in interstitial bone and did not encounter secondary osteons (b) microcracks which initiated in interstitial bone but, when they encountered secondary osteons, stopped growing outright (c) microcracks which initiated in interstitial bone and, when they encountered secondary osteons, continued to grow and were deflected around the cement line but did not propagate into the circumferential lamellae of the osteon (d) microcracks which initiated in interstitial bone and, when they encountered secondary osteons, penetrated the cement line and propagated into the osteon and (e) microcracks which encountered secondary osteons at both tips of the crack and did not propagate at either end. Microbar= 100 μm in each image.

Fig. 3. Mean crack length at the time of encountering an osteon for each of the five categories. One-way analysis of variance (ANOVA) showed that crack length at the time of encountering an osteon significantly affected its ability to propagate ($p<0.05$). Pairwise multiple comparison procedures showed individual statistical differences ($p<0.05$) between all groups except (i) groups 1 and 3 and (ii) groups 2 and 5.

Fig. 4. This illustrates a typical example of two large cracks (white arrows) that were involved in failure of a specimen. It can be seen clearly that as these cracks grew to macrocrack size, they managed to penetrate the cement lines of a number of osteons and in some cases propagated directly through the Haversian canals (black arrows). Much secondary damage related to the main cracks is evident in this figure. However evidence of crack arrest at cement lines is also present (dashed arrows). Microbar= 100 μm.

Table 1. Criteria for identifying microcracks in bone [17,21,22].