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Bone as a composite material:

the role of osteons as barriers to crack growth in compact bone

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ABSTRACT

This article summarises a number of studies in the area of bone microdamage which were carried out in our laboratory over the past 5 years. A technique was developed to label microcracks during mechanical testing. Fluorescent chelating agents were applied at intervals to bone specimens fatigue tested in cyclic compression until failure occurred. Microcrack densities were measured and microcrack length at the time of encountering the cement line surrounding an osteon was also recorded. Microcracks were shown to develop rapidly during the first stage of testing but then further accumulation of cracks did not occur until the period just before failure. The majority of microcracks were found in interstitial bone and did not penetrate cement lines. Only microcracks greater than 300μm in length were found to be capable of penetrating osteons. This work provides experimental data to support the hypothesis that secondary osteons act as barriers to crack propagation in compact bone.

1 INTRODUCTION

Fatigue damage in bone occurs in the form of microcracks due to the regular day to day activities of normal life in healthy human beings. This damage acts as a stimulus for bone remodelling (Martin and Burr [1], Burr et al. [2], Burr and Martin [3], Mori and Burr [4], Lee et al. [5], Martin [6], O'Brien et al. [7]). Bones, therefore, have an advantage over most engineering structures in that they have an inherent ability to repair damage. However if this damage accumulates at such a rate that the capacity for bone repair is exceeded, stress fractures result. These fractures occur commonly in athletes and soldiers engaged in high intensity, repetitive activities such as marching or running. If, on the other hand, damage accumulates at 'normal' rates but

the bone's repair mechanism is deficient, fragility fractures result which occur commonly in osteoporotic bone (Diab et al. [8]; O'Brien et al. [9], Schaffler et al. [10]).

Secondary osteonal bone has been compared to a composite material and to metals whereby microstructural features within the material, such as laminae and voids, may provide sites for crack initiation, but they also serve as barriers to crack growth which may slow down or even halt crack propagation completely. It has been proposed that a microstructural barrier concept may exist in bone (Martin and Burr [11], Taylor and Prendergast [12], Akkus and Rimnac [13]; Sobelman et al. [14]) whereby the microstructure of osteonal bone provides barriers to crack growth in the form of cement lines which surround secondary osteons. The cement line interface between osteons and interstitial bone is relatively weak which means that it may reduce the shear strength of osteonal bone (Frasca [15]). However it has been hypothesised that slipping at this interface relaxes shear stresses, reducing strain energy and thus slowing crack propagation. Jepsen et al [16] showed that the lamellar interface in bone is weak and is the principal site of shear damage formation but the lamellar interface was shown to be highly effective in keeping cracks isolated from each other. Zioupos et al. [17] showed that microcracks in bone did interact with the microstructure of the bone and that the grain of the bone constrained their growth directions. They hypothesised that the presence of lamellae influenced the process by which microcracks coalesced but that vascular or other naturally occurring cavities did not initiate microcracking and appeared to deflect microcracks. Work by Schaffler et al. [10] added quantitative data to this hypothesis suggesting that 80-90% of all microcracks in cortical bone are found in the interstitial matrix between osteons.

This article summarises a number of studies which were carried out in our laboratory over the past four years (O'Brien et al. [18-20]. One of the challenges with studying microcracks in bone is developing a technique with which to monitor them. Lee et al, [21] demonstrated that the application of fluorescent chelating agents in sequence could be used to monitor microcrack growth *in vitro.* These agents are as effective as the standard method, basic fuchsin, in identifying microcracks but are also 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. However substitution of one agent by another due to varying affinities for exposed calcium which line the walls of microcracks made measuring crack growth imprecise. This paper summarises how the method of detection was refined in order to determine the optimal sequence of application for five chelating agents which allowed all the agents to fluoresce equally brightly using UV epifluorescence and avoided substitution. Following development of the optimal labelling technique, it was proposed to label microcracks and monitor microcrack development during fatigue testing to look at the process by which microcracks propagate and interact with the bone's microstructure ultimately bringing about failure. Furthermore, the authors sought to test the hypothesis that bone behaves as a composite material and, if so, to determine the components of the bone's microstructure which allow this comparison to be made. In particular, we wished to determine whether cement lines influenced crack growth and whether a microstructural barrier phenomenon exists in bone.

2 MATERIALS AND METHODS

2.1 Development of an optimised labelling technique

The aims of this experiment were to refine the method of detection developed by Lee et al. [21] in order to determine the optimal sequence of application for the five fluorescent chelating agents which allowed all the agents to fluoresce equally brightly using UV epifluorescence and avoided substitution. The levels of free calcium in a solution of calcium chloride before and after the introduction of each chelating agent were measured using ion chromatography (Haddad and Jackson, [22]). The calcium chloride concentration was 1 x 10^{-3}M and each of the five chelating agents, alizarin complexone (A), calcein blue (B), xylenol orange (X) (all from Aldrich Chemical Co., Milwaukee, Wi., USA), calcein (C) (Sigma Chemical Co., St. Louis, Mo., USA) and oxytetracycline (O) (Bimeda Ltd, Dublin) was injected separately at a concentration of 5 x 10^{-4}M. The chelating agents were ranked in order of decreasing affinity for calcium and this sequence was then tested on bone specimens using a scratch test technique. Samples of cortical bone were removed from the mid-diaphysis of bovine tibiae and machined into beam-shaped specimens using a band saw. These were then finely polished using emery paper. Using a compass point, a 5mm straight line was scratched on the upper surface of the bone beams. Each specimen was immersed in a vial containing a 5 x 10^{-4}M aqueous solution of the fluorescent agent and placed in a vacuum desiccator for 4 hours. The specimen was washed in de-ionised water, a second 5mm line scratched parallel to the first and the specimen immersed in a vial containing a 5 x 10^{-4}M aqueous solution of a second fluorescent agent and placed in the vacuum desiccator for 4 hours. This protocol was repeated using two, three and four dye sequences. Following establishment of the optimal sequence of application,

mechanical tests were carried with the fluorochromes applied in sequence to monitor crack growth.

2.2 Mechanical testing

Samples were taken from fresh bovine tibiae and machined into typical, waisted, "dog-bone" type specimens of circular cross section using an established protocol (Taylor et al [23]). 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, and at a stress range of 80 MPa (between 8 and 88MPa). The fluorescent chelating agents were applied in the pre-determined sequence in order to label microcracks formed prior to, and during the tests. Initially, the machined specimens were placed in a single vial of the first agent in a dessiccator under vacuum for 16 hours to label any microdamage which existed prior to testing. Testing was carried out with the second agent for the first 10,000 cycles of testing. The test was stopped, the bath was rinsed with distilled water and the third agent added. Testing was continued until 50,000 cycles had elapsed and the fourth agent applied. As explained in the Results and Discussion sections of this paper, difficulties were encountered with the use of all five agents in sequence and therefore only four agents were used. Failure was defined using established criteria [23]; a 10% reduction in stiffness which generally coincided with the appearance of a large crack.

2.3 Microcrack analysis

Following testing, the gauge length of the specimens was removed using a diamond saw (Struers Miniton). Sections 250 μm thick 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 the established criteria (Lee at al. [21,24], O'Brien et al. [25]). Numerical crack density: Cr.Dn (number of cracks occurring per mm^2) was also measured. Microcracks were initially classified into two categories: osteonal, where they were located entirely within a secondary osteon or traversed a cement line surrounding a secondary osteon, and interstitial: where they were located completely in interstitial bone and did not penetrate the cement lines surrounding these osteons.

Microcracks which did encounter osteons were further classified into three distinct categories: (i) microcracks which initiated in interstitial bone but when they encountered secondary osteons, they stopped growing outright; (ii) microcracks which initiated in interstitial bone but when they encountered secondary osteons, they continued to grow but their path was deflected around the cement line surrounding the osteon and they did not propagate into the circumferential lamellae of the osteon; (iii) microcracks which initiated in interstitial bone but, when they encountered secondary osteons, penetrated the cement line and propagated into the osteon. The relationship between microcrack length and location using the three distinct categories was then analysed.

3 RESULTS

The results from the ion chromatography analysis demonstrated that alizarin complexone had the greatest affinity for calcium followed by xylenol orange (X), calcein blue (B), calcein (C) and oxytetracycline (O). Scratch tests were carried out using chelating agents applied in this order. However the scratch test analysis led to a revision in this sequence as C was found to have a greater affinity for calcium than B i.e B followed by C resulted in greater fluorochrome substitution than C followed by B. The concentration of B was then reduced in stages, firstly to 2.5 x 10^{-4} M and then to 1 x 10^{-4} M until the degree of substitution was negligible. O was problematic as it tended to substitute each of the other agents regardless of the sequence of application. This could not be rectified by altering the sequence or concentration and so O was excluded from the study. The revised four stain protocol was A-X-C-B, using 5 x 10^{-4} M concentrations of A, X and C and B at 1 x 10^{-4} M. Figure 1 demonstrates this sequence using an image from the scratch test analysis. All four scratched regions can be clearly distinguished from each other and from the surrounding bone matrix and substitution is negligible. This sequence was then used during mechanical testing.

The fluorescent chelating agents allowed a clear distinction to be made between pre-existing microcracks and microcracks formed at individual periods during testing. Only 6% of microcracks were pre-existing and these did not propagate during testing. Figure 2 illustrates the pattern of microcrack accumulation (crack density) during the course of a test. Microcracks were shown to develop rapidly during the first 10,000 cycles, but no significant increase took place between 10,000 cycles and 50,000 cycles. A further increase in microcrack- density then took place between 50,000

cycles and failure with the mean N_f being 88,380 (S.D. 22,400) cycles to failure. No significant difference was found in length between microcracks which were found exclusively in a single period during testing and which did not propagate beyond this period (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 were short in comparison to the other types (56 µm S.D. 50 µm), were found close to the surface of the specimen and were not found to propagate during testing. One of benefits to using this sequential labelling technique is that microcracks which had propagated between two or more distinct periods of testing were labelled with two or more dyes and therefore allowed them to be distinguished from cracks which had been formed during a single period of the test. These propagating microcracks were found to be longer than microcracks formed at individual periods during testing (281 µm S.D. 119 µm).

The majority of microcracks were located in interstitial bone (85%) and did not penetrate the cement lines surrounding secondary osteons. Figure 3 shows the analysis of those cracks which did encounter cement lines. One-way analysis of variance (ANOVA) showed that there was a significant difference between all groups ($p<0.05$), indicating that crack length at the time of encountering an osteon significantly affected its ability to propagate. This illustrates the influence of crack length on its ability to penetrate cement lines and propagate through an osteon. The first category indicates that if cracks were less than 100 µm (mean: 95 µm S.D. 26 µm) when they encountered a cement line surrounding an osteon, they stopped growing outright. However the mean length for cracks which were deflected when they encountered the cement line was 174 µm (S.D. 47 µm). A general observation,

albeit one for which we have no data, showed that these deflected cracks generally stopped growing soon after the encounter with a cement line and were not observed to become macroscale cracks. The third category shows cracks which did actually manage to penetrate one or more osteons; these were significantly longer ($p<0.05$) than the other categories (mean: 313 μm S.D. 116 μm).

4. DISCUSSION

This study describes how a technique, using fluorescent chelating agents to sequentially label microcrack growth in bone was developed (Lee et al. [21]) then refined (O'Brien et al. [18]) and subsequently used, by applying the agents at different intervals during a mechanical fatigue test, to learn more about microcracks, their effect on the fatigue behaviour of bone, their interaction with the bone's microstructure and the processes by which they initiate and grow (O'Brien et al. [19,20]). The optimal sequence of application and concentration of each agent was alizarin complexone (0.0005M) followed by xylenol orange (0.0005M), calcein (0.0005M) and calcein blue (0.0001M). A fifth agent, oxytetracycline was excluded from the study after recurring problems were found with its ability to chelate exposed calcium when applied in sequence with the other agents. Crack accumulation during the life of a test specimen followed a characteristic curve in which many cracks initiate early during the specimen's life (first 10,000 cycles) but then accumulation of more cracks is suppressed with only a slight increase occurring between 10,000 and 50,000 cycles before microcracks rapidly accumulate after 50,000 cycles eventually resulting in failure. It has been proposed that a microstructural barrier concept governs the fatigue behaviour of bone whereby the microstructure of secondary

compact bone allows microcracks to initiate rapidly but because of the morphology of osteonal bone, microcracks encounter barriers which suppress further growth until late in a bone's life. If the crack accumulation characteristics found in this study are compared to those for composite materials (Figure 4) a similar trend is found; in fibreglass, cracks initially begin to grow quite easily in the matrix but then they meet the fibres which act as barriers and prevent further growth. This means that although a composite material may provide numerous sites for crack initiation, it is also a relatively tough material as cracks find it difficult to propagate to critical lengths.

In this study, the vast majority of microcracks were found in interstitial bone. Figure 3 shows the relationship between microcrack length and their ability to grow. Microcracks shorter than 100 μm in length were likely to stop growing if they encountered an osteon while cracks in the range 150-300 μm may continue to grow after encountering cement lines surrounding secondary osteons but they are likely to be deflected and often cease growing soon afterwards. Only microcracks greater than 300 μm in length when they encounter osteons were shown to have any real potential to grow to critical lengths and cause failure. No significant difference in length was found in microcracks formed at individual periods during testing, however, propagating microcracks which grew during at least two stages of the specimen's life were found to be significantly longer ($p<0.05$) than microcracks formed at individual periods during testing which did not continue growing in a later stage during the test. This length is similar to that of microcracks which penetrated cement lines, indicating that microcracks which were formed at least as early as the second stage of the specimen's life, and then continued to propagate, had a greater chance of breaking through osteons.

Failure was observed to occur with the propagation of one, or very few, long cracks to critical lengths, rather than by the coalescence of numerous small microcracks. However, an interesting observation was that these cracks always penetrated a cement line at some stage on the path to failure. Figure 5 shows a typical example of two large cracks 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 numerous osteons. This was a recurring theme when the fracture surfaces were studied, all failure surfaces showed splitting of osteons usually at the Haversian canals in the centre. These canals provide vascular supply to the bone tissue and are therefore essential to the well-being of the bone tissue. However, as failure tended to occur with the critical growth of cracks which had penetrated cement lines, rather than the growth of cracks which were found in regions of the bone with few secondary osteons, this would suggest that, in the event of microcracks growing to lengths which allowed them to penetrate cement lines, these canals acted as weaknesses in the bone and allowed a pathway for further propagation and eventual failure.

5. ACKNOWLEDGEMENTS

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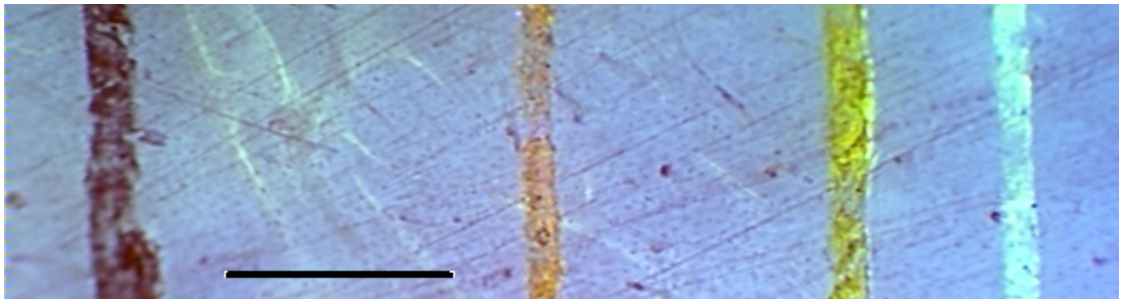

Fig. 1 The optimal four agent sequence developed from the scratch tests, from left to right: alizarin complexone (0.0005M), xylenol orange (0.0005M), calcein (0.0005M) followed by calcein blue (0.0001M). All 4 stains are clearly distinct from each other and from the surrounding bone. Scale bar=200 µm.

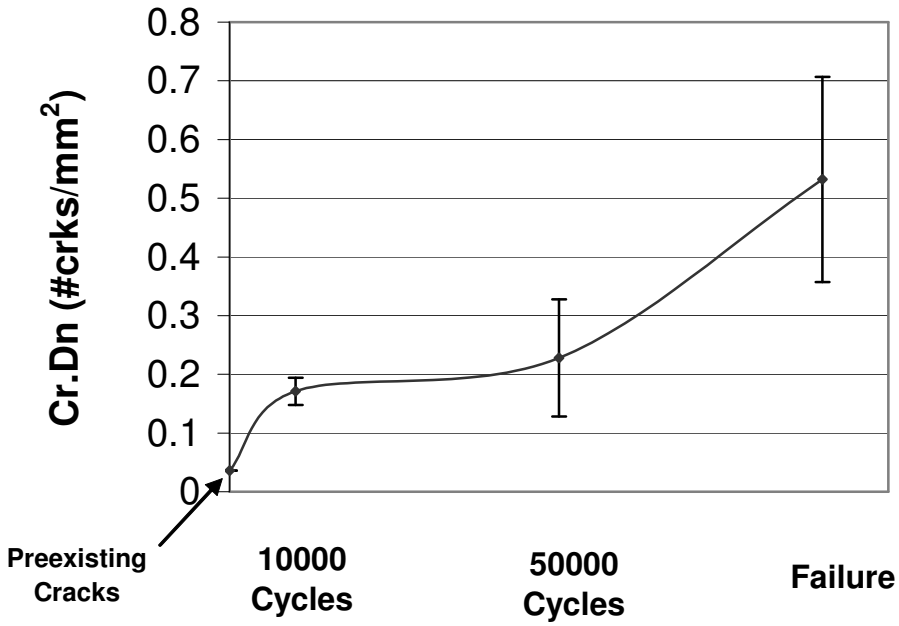

Fig. 2 Accumulated microcrack density v time. Microcrack density increased rapidly during the first 10,000 cycles but then there was a reduced rate of accumulation until 50,000 cycles have elapsed after which there is another rapid rate of accumulation.

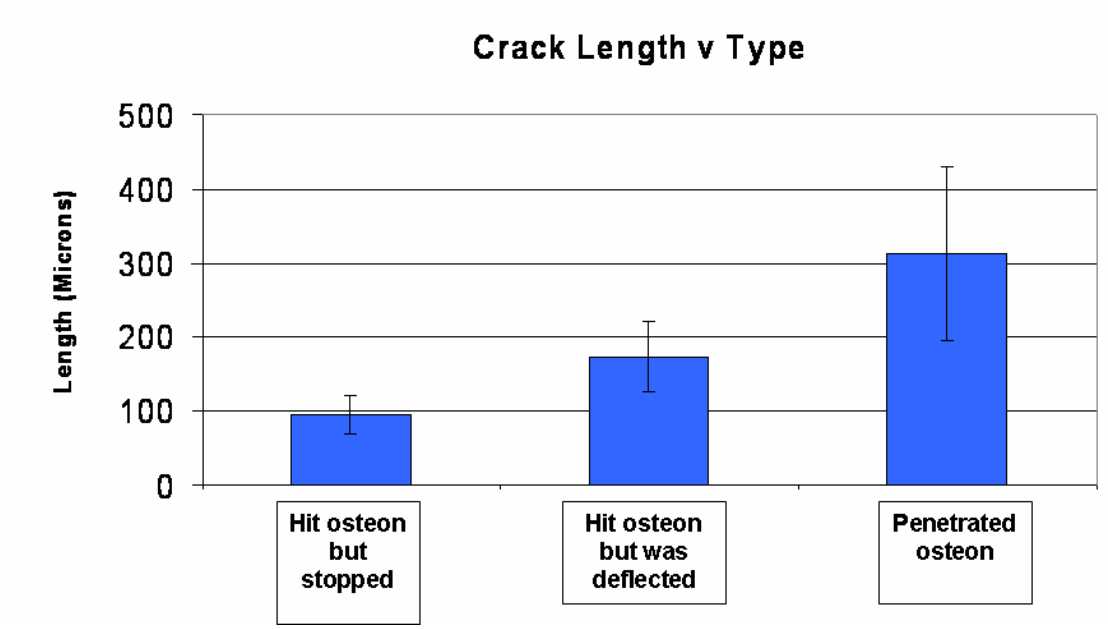

Fig. 3. Mean crack length at the time of encountering an osteon for each of the three categories. One-way analysis of variance (ANOVA) showed that crack length at the time of encountering an osteon significantly affected its ability to propagate ($p<0.05$).

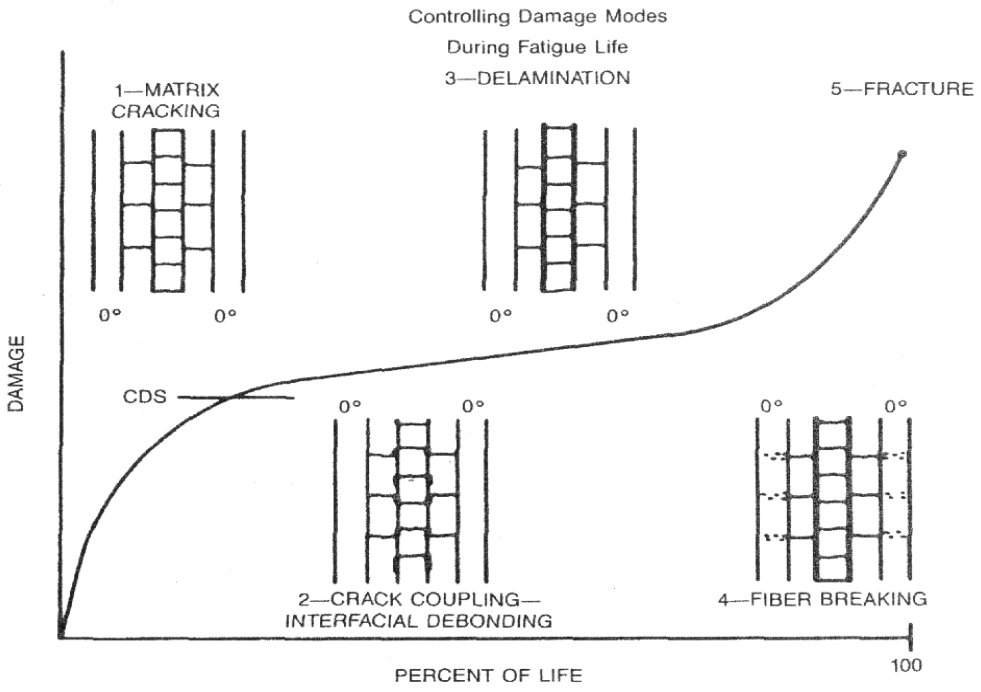

Fig. 4 Damage accumulation in composite materials. Cracks initially grow quite easily in the glass matrix but then encounter barriers to further growth in the form of the fibres running through the material (Reifsnider et al. [26]).

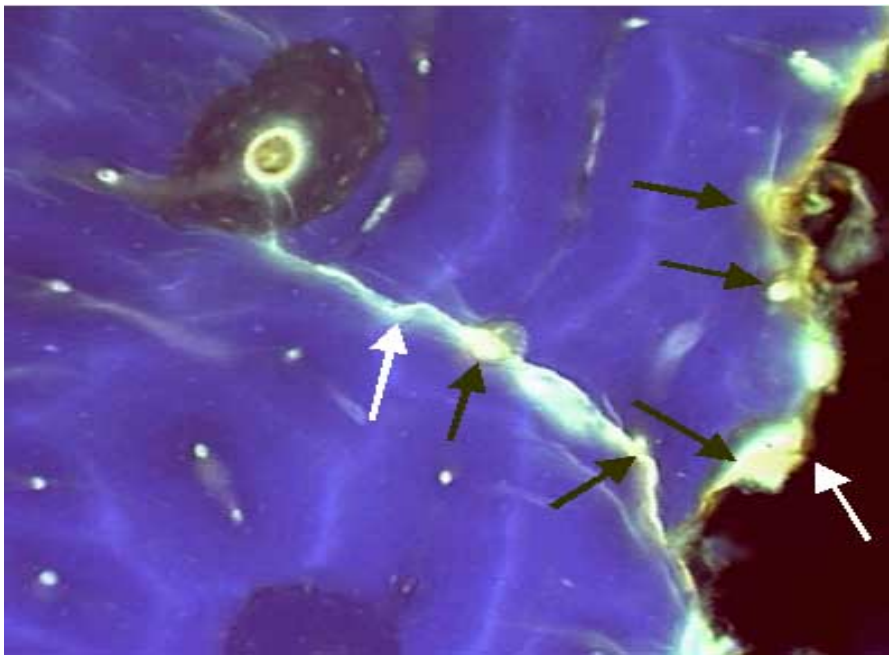

Fig. 5. This illustrates a typical example of two large cracks (white arrows) that were involved in failure of a specimen (the second crack is the main failure surface). As these cracks grew to macrocrack size, they managed to penetrate the cement lines of numerous osteons and used the Haversian canals (black arrows) as weaknesses in which to further propagate.