Collagen fibre architecture in the periodontal ligament[1]

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According to a recent review published by the Medical Research Council (1977), periodontal disease is the major cause of tooth loss in adult life. Despite the enormous incidence of periodontal disease, many aspects concerning the basic structure and properties of the attachment tissues of the teeth remain unclear. The question of the nature of the arrangement of the collagen fibres in the periodontal ligament has vexed dental histologists for many years.

Careful descriptions of the periodontal ligament were published by Black (1887, 1899), who considered that the periodontal collagen was organized into principal fibre bundles which passed directly from tooth to bone and which supported the tooth rather in the fashion of a sling. Sicher (1923, 1942) proposed that the principal fibre bundles did not pass directly from tooth to bone but that instead they formed a spliced arrangement in the middle of the ligament, most clearly defined in continuously erupting teeth. Many authors have questioned the existence of the intermediate plexus (*see* Zwarych & Quigley 1965) and Ciancio *et al.* 1967 suggested that it was a histological artefact arising as a result of the section plane lying oblique to the principal fibre bundle direction. Recently, a technique which allows satisfactory examination of the periodontal ligament in the scanning electron microscope (SEM) has been developed (Sloan *et al.* 1976) and has been used to examine the fibrous architecture of the periodontal ligament in some teeth of continuous growth (Sloan 1978). It was considered that this method might be employed to examine the arrangement of the periodontal collagen in some teeth of limited growth, where the ligament resembles that of man histologically.

Methods

The material examined was derived from two macaque monkeys, *Macaca fascicularis*, with a full adult dentition. The jaws were fixed by perfusion with 10% buffered formalin, defleshed, and blocks containing the incisor, canine and premolar teeth respectively were excised with a cooled, rotating abrasive disc and stored in fresh 10% buffered formalin until required.

Maxillary and mandibular blocks from the left side of both animals were placed into 10% EDTA (ethylene diamine tetra-acetic acid) at pH 7.3, which was continuously stirred. When demineralization was complete, as judged radiographically, the blocks were rinsed in 0.2 mol/l phosphate buffer and sectioned with a honed, degreased razor blade in either the longitudinal (buccolingual) or longitudinal (mesiodistal) plane. Some blocks were then subdivided with a series of cuts made in the transverse plane. The blocks were treated with crude bacterial α-amylase (BDH Chemicals Ltd, Poole, Dorset) to remove ground substance, using the method described by Hunter & Finlay (1973), and were subsequently critical point dried in ethanol and coated for examination in the SEM, using the methods described by Sloan *et al.* (1976).

The right maxillary and mandibular blocks from both animals were dehydrated by refluxing with a 1:1 chloroform/methanol mixture in a Soxhlet apparatus over a period of 48 hours. They were then infiltrated with methyl methacrylate containing 1% benzoyl peroxide, which polymerized in daylight in two weeks. Ground sections retaining the soft tissues were prepared with a Microslice II slitting machine (Cambridge Instruments Ltd, Cambridge, England) set to cut at 100 μm, in the transverse and longitudinal (buccolingual) planes. The sections were polished to a final thickness of approximately 80 μm with 600 grade abrasive, cleaned ultrasonically and, following dissolution of the methacrylate with chloroform, mounted unstained in Canada balsam for examination in the polarizing microscope.

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Results

At low magnification in the SEM, the dentine, cementum, periodontal ligament and alveolar bone could be distinguished clearly. The periodontal ligament appeared to be a densely packed, collagenous tissue, 100–150 µm in width, and contained longitudinally-orientated blood vessels. The collagen appeared to be organized into approximately circular bundles, most of which were 3–8 µm in diameter. In all the sections studied, the bundles always exceeded 1 µm in diameter and in longitudinal (axial) and transverse sections, profiles of bundles which had been sectioned in a variety of planes were evident (Figure 1). The bundles pursued a complex, wavy course and branched and anastomosed frequently forming a network. The bundles coursed around the blood vessels and because of the frequent branching it was impossible to trace a single bundle across the periodontal space. Distinct bodies, possessing flattened sheet-like processes, were evident lying between the bundles (Figure 1); these were interpreted as fibroblasts. In certain longitudinal (tangential) sections, the bundles appeared circular and the fibroblasts were triangular or stellate in outline, and possessed extensive processes. These partly or completely surrounded the bundles (Figure 2). Close to the alveolar wall, the bundles were 10–15 µm in diameter (Figure 2), although they rapidly divided into the smaller bundles which made up the majority of the ligament.

In ground sections viewed in the polarizing microscope, the periodontal ligament appeared to be composed of a network of birefringent collagen fibre bundles. In the 90° position, the bundles in transverse section appeared to be approximately radially orientated, although they curved around the blood vessels, which appeared as dark areas (Figure 3). However, in the 45° position, overlapping groups of bundles having two distinct oblique orientations became visible (Figure 4).

These groups of bundles did not follow the shortest course between tooth and bone. In some places, sharply defined, periodic extinction lines were observed and when a gypsum plate (red I) was inserted between the specimen and the analyser, the collagen on either side of each extinction line showed an opposite sign of birefringence. In longitudinal sections, crestal, horizontal, oblique and apical groups of fibre bundles, such as those described by Black (1899), were observed. The bundles were birefringent, and in places overlapping groups of bundles of different obliquity were found. Periodic extinction lines similar to those found in transverse sections were observed only in some parts of the longitudinal sections.

Discussion

The SEM observations showed that the majority of the periodontal collagen was organized into highly orientated, circular bundles which were arranged into a network. No spliced arrangement, or intermediate plexus, of the kind described by Sicher (1923, 1942) was

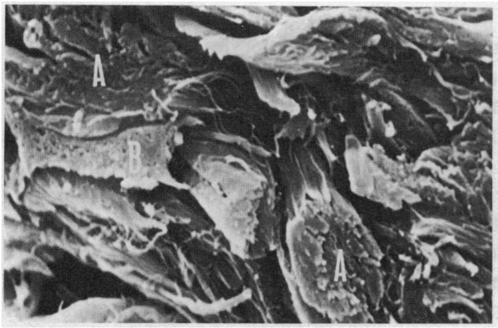

Figure 1. Longitudinal (axial) section of periodontal ligament in the mid-root region of an upper premolar, viewed in the SEM. Profiles of collagen bundles (A) and a fibroblast (B) are evident (× 2500)

Figure 2. Longitudinal (tangential) section of periodontal ligament in the mid-root region of an upper premolar, viewed in the SEM. Transversely-sectioned, branching collagen bundles (A) separated by fibroblasts are evident (× 1000)

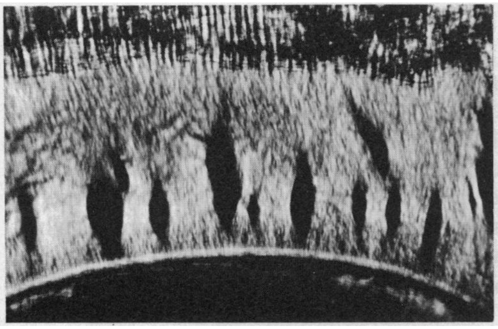

Figure 3. Transverse ground section of the periodontal ligament of an upper premolar viewed in the polarizing microscope in the 90° position. The appearance is of radially orientated fibre bundles (× 125)

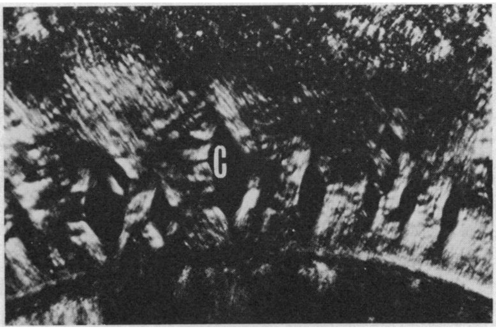

Figure 4. Same field as Figure 3, viewed in the 45° position. Overlapping groups of bundles are now evident and crimping can be seen at C. (× 125)

observed. The findings directly contradict those of Shackleford (1971, 1973) and Svejda & Skach (1973) who, on the basis of SEM observations, considered that the majority of the periodontal collagen was randomly orientated, forming an indifferent plexus.

The intermediate and indifferent plexuses were proposed to account for the maintenance of tooth support when ligament remodelling occurred during tooth movement. Studies of collagen turnover using autoradiography (Carniero & Fava de Moraes 1965, Rippin 1976) suggest that ligament remodelling occurs rapidly and is not localized to any particular layer within the ligament. In association with this rapid remodelling, Ten Cate (1972) proposed that fibroblasts may phagocytose and degrade, as well as synthesize, collagen and intracytoplasmic collagen profiles have been reported in periodontal fibroblasts in many situations (Listgarten 1973, Ten Cate & Syrbu 1974, Garant 1976, Frank *et al.* 1976). The present results demonstrated a close relationship between the fibroblasts and the collagen, the collagen bundles being partly or completely surrounded by cell processes. Such an arrangement would allow access to all parts of the collagen bundles for cellular degradation. The complex path and wavy course pursued by the bundles may, however, be of importance in maintaining tooth support.

The overlapping groups of bundles seen in the polarizing microscope (Figure 4) suggested that there was a criss-cross arrangement of the ligament collagen. This pattern would have certain mechanical advantages over a simple radial arrangement of the bundles. On the basis of physical measurements, Minns *et al.* (1973) suggested that collagenous bundles in many connective tissues, e.g. tendon, could best resist forces which were axially directed. The criss-cross pattern would allow forces tending to rotate the tooth to be transmitted axially along the ligament bundles. A similar criss-cross arrangement is often used in mechanical engineering, for instance, in arranging the spokes of a wheel.

In the present study, a zigzag arrangement of the collagen was observed in the polarizing microscope at high magnifications. Diamant *et al.* (1972) and Keller & Gathercole (1976) described a similar arrangement in rat-tail tendon and proposed a structural model in which units of collagen were considered to be joined by a series of rigid hinges. This crimped structure could be correlated with the stress-strain curve for rat-tail tendon, which shows an early, nonlinear region of easy extensibility, followed by a linear region. Keller & Gathercole (1976) noted that most physiological stresses were within the nonlinear region, where the easy extensibility is considered to be due to straightening out of the crimp. Recently periodic crimping, similar to that in rat-tail tendon, has been observed in teased preparations of human periodontal ligament (L J Gathercole, personal communication) where the fibre bundles tend to orientate themselves approximately at right angles to the optical path. The criss-cross arrangement of the bundles observed in the ground sections (Figure 4) might explain why crimping is seen only in some parts of a section and not in others. Any ground section would be expected to contain bundles having a variety of orientations, and crimping would only be evident in those bundles which were approximately normally orientated to the optical path.

In a detailed study of the physical properties of the periodontal ligament, Wills *et al.* (1976) showed that fluid systems were most important in supporting teeth when subjected to forces below 1.0 N, but that the fibrous components were directly involved in transmitting forces of greater magnitude. Intrusive forces encountered in mastication are believed to be greater than 1.0 N (Parfitt 1967), and such forces would tend to straighten out the crimp. The overlapping arrangement of the bundles would mean that this effect could operate over a greater length than if a simple radial arrangement was present. Straightening out of the bundles by intrusive forces might also compress adjacent blood vessels. These changes might be important in producing the viscoelastic properties of the periodontal ligament noted by Parfitt (1967).

Evidence from clinical, radiological and histopathological studies (Cohen 1959) suggests that periodontal disease originates coronally and spreads apically. Difficulty exists, however, in separating possible age-related changes from pathological changes in the ligament which survives. In rat-tail tendon, there is progressive flattening and lenthening of the crimp with age (Keller & Gathercole 1976) and further measurements of crimp length and angle in periodontal collagen might be worthwhile, as any variations are likely to reflect age, rather than pathological changes. In any event, the crimped nature and complex, three-dimensional, overlapping arrangement of the collagen bundles should be taken into account in advancing any hypothesis of the mechanism of tooth support.

Summary

The arrangement of the collagen in the periodontal ligament of the macaque monkey was investigated using polarizing microscopy and scanning electron microscopy (SEM). The collagen fibres were found to be grouped together to form circular bundles, which were organized into a branching network connecting tooth to bone. The collagen within individual bundles showed a zigzag, or crimped, arrangement and the networks formed a complex, three-dimensional, overlapping array. In the SEM, fibroblasts possessing extensive, flattened processes which partly or completely surrounded the bundles, were frequently encountered. A model of the arrangement of the cells and collagen in the periodontal ligament is proposed and its significance in understanding the mechanism of tooth attachment is discussed.

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