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Depèndence of rate of physical erosion on orientation and density in mineralised tissues

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Summary. Bone, dentine and enamel samples were treated with a gas-propelled jet of an abrasive, $NaHCO_3$, which is physically much softer than any of these tissues in their fully mineralised condition. It was nevertheless found that they are all eroded by this treatment, which can therefore be used as a new kind of qualitative test of physical properties relating to wear resistance. General correlations were found between both degree of mineralisation and between structure orientation and erosion rate, surface-parallel-feature zones being worn more rapidly. Bone domains with surface-parallel collagen were eroded faster than those with perpendicular lamellae even if they were more densely mineralised. Rates of dentine wear depended on both density and tubule orientation, with peritubular zones and better mineralised incremental layers being more resistant. Enamel tufts wear more rapidly than the surrounding well mineralised regions. Enamel diazones wear less than parazones (areas with surface parallel prisms). At the prism scale, enamel is removed more rapidly near prism boundary discontinuities and in tubular enamel, at tubule walls. As regards the common orientation dependent effects seen in these three tissues, a cohesive explanation would be that structure discontinuities can be better exploited in a wear process if they allow cleavage from the surface; which tendency will increase with parallelism to the surface.

Key words: Airpolishing – Orientation effects – Enamel – Dentine – Bone

Introduction

It is probably obvious that the rate of removal of a mineralised tissue undergoing any kind of wear or attrition process, whether due to normal functional use or in grinding and polishing in specimen preparation for microscopy, would vary with the tissue density, since hardness would be expected to increase with the degree of mineralisation. Differences in the rate of wear may, however, also relate to the direction of the abrading or eroding factor with respect to structural direction.

At the ultrastructural scale, these factors have been shown to be operative in the atomic or ion beam erosion (micro-milling) process in which sections can be thinned in preparation for transmission electron microscopy (TEM) (Boyde 1974), or surface etched for scanning electron microscopy (SEM) (Boyde and Stewart 1962). Thus the more highly mineralised peritubular dentine zones stand proud of an ion etched surface, but within the comparatively uniformly densely mineralised enamel, etching occurs due to differences in crystal orientation (Boyde and Stewart 1962).

At a much grosser scale, dentine is eroded more rapidly than the more highly mineralised enamel under ion beam erosion and the less mineralised tuft regions within enamel more than the surrounding tissue. Osborn (1965) brought into prominence the fact that the Hunter-Schreger bands in enamel, which depend upon the existence of zones with contrasting prism orientations, are brought into etch relief by the grinding and polishing processes of section preparation. Rensberger and von Koenigswald (1980) have shown that the vertical Hunter-Schreger bands in rhinoceros enamel are strongly etched by the airbrasive technique in which hard abrasive particles are propelled in a dry, high velocity gas stream.

The purpose of this paper is to report the finding that treatment of mineralised tissues with an airpropelled stream of *soft* abrasive particles also causes physical erosion of the mineralised tissues which varies with density and orientation dependent structure factors. The method used is the technique trade-named "airpolishing" in which the abrasive used is sodium bicarbonate, which is much softer than any of the mineralised tissues. The particle stream is surrounded by a concentric water jet which wets and dissolves the bicarbonate so that the "abrasive" is further softened outside the focal centre of the stream. This method can be used to study, qualitatively, variations in the microhardness of the mineralised tissues, and can involve minimal damage to the untreated parts of a bone or tooth sample.

Materials and methods

Enamel, dentine and cement were studied in mature teeth of man, African elephant, white rhinoceros, black rhinoceros, sperm whale, kangaroo, koala bear, domestic cattle, horse and rat stored dry or in 70% ethanol. Immature, partly mineralised enamel was studied in pig, calf and rat teeth used fresh or after fixation and storage in 70% ethanol.

Bone samples were obtained from human, macaque monkey, and dog material stored in 70% ethanol. All samples were re-equilibrated with water before use.

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Natural surfaces, wear surfaces and oriented, cut and polished surfaces were treated with the abrasive stream from a "Dentsply®-Cavitron® PROPHY-JET® Airpolishing Prophylaxis Unit" (Dentsply/York Division, Dentsply International, York, Pennsylvania 17405, USA), using an air input pressure of 65–80 PSI and water input pressures of 25–60 PSI, with Prophy-Jet® cleaning powder-1 (Cavitron Division of Cooper Medical Devices Corporation, 11-40 Border Ave, L.I. City, NY 11101, USA).

All treatments were carried out at working distances of 3–5 mm under observation with a stereobinocular microscope. To describe the "dose" received, the treatment periods were timed, and the area over which the beam was moved was recorded. The rate of removal could be assessed by covering a portion of the surface with a razor blade edge or a metal aperture. The depth of tissue removed could then be measured by tilting the specimen in the SEM to view the resulting step feature on edge.

After airpolishing treatment, the samples were washed with water, dehydrated with ethanol, air dried from $C_2Cl_3F_3$, and coated with Au:Pd by sputtering. They were examined in a Cambridge Stereoscan S4-10 SEM operated in the secondary electron emission mode (SE), converted backscattered electron mode (CBSE), the backscattered electron mode using a solid state annular detector (BSE), or an Everhart-Thornley biassed scintillator detector with the grid biassed to −50 V to prevent the collection of low energy secondary electrons (BSE-ET).

Results

Relative airpolishing abrasion rates in the different hard tissues

Comparing the rates at which the different mineralised tissues were eroded, there was an overall correlation between hardness and resistance to airpolishing effects. The most rapidly eroded material was immature dental enamel, followed by cement and bone, then dentine, with mature enamel as the most resistant material studied.

Enamel

The results obtained with human enamel have significance for operative, clinical technique and are described fully elsewhere (Boyde 1984).

For each species studied, it was found that selective resistance to airpolishing erosion was demonstrated both by the enamel at the enamel-dentine junction (EDJ) and at the true enamel surface (TES). Both of these zones lack the prism boundary discontinuities found throughout the bulk of the enamel tissue, which not only was eroded, but developed a regular and interpretable etch relief as a consequence.

The resistance to erosion of the EDJ and TES could be exploited to prepare pure enamel samples. Thus, for example, deposits of calcified bacterial plaque (calculus) could be removed from erupted, mature human tooth samples, leaving the enamel surface clean and intact. Coronal cementum was removed from koala teeth, exposing enamel. Similarly, by removing the bulk of dentine from the inside of the enamel shell of koala and kangaroo teeth using conventional dental burs, it was possible to remove the dentine residue, to expose the EDJ surface of the enamel by "airpolishing" erosion. Such preparations of marsupial teeth make it possible to visualise the distribution of the enamel tubules (Tomes 1849; Boyde and Lester 1967) at both surfaces of this tissue (Figs. 1, 2).

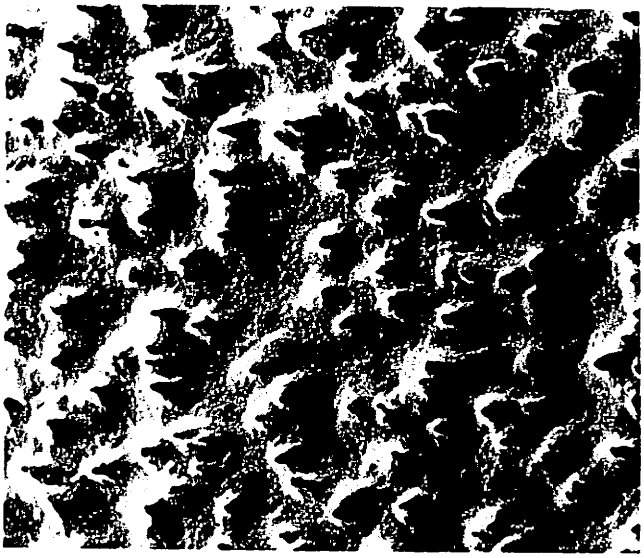


Fig. 1. Kangaroo (*Macropus sp.*), enamel side of enamel-dentine junction: dentine removed by airpolishing. Holes are enamel tubules 10 kV SE. Fieldwidth 85 µm

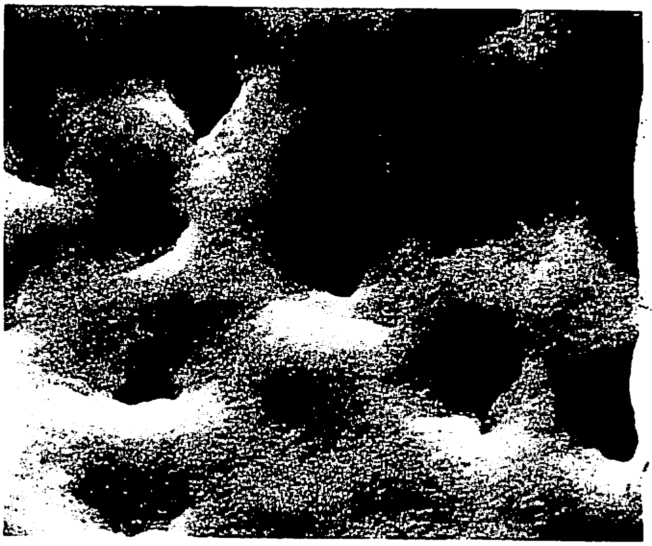


Fig. 2. Koala (*Phascolarctos cinereus*), surface of occlusal enamel from which coronal cementum has been removed by airpolishing. Enamel tubules can be identified in the ameloblastic Tomes process pits. 20 kV SE. Fieldwidth 25 µm

Etching – the development of surface topographic relief due to differences in the rate of removal of the pre-existing surface layers which were removed – in enamel occurred in relation to several classes of structure.

(A) Tufts were removed more rapidly than the surrounding enamel, presumably due to their lower degree of mineralisation.

(B) Enamel tubules in the marsupial species were enlarged after airpolishing, presumably because microscopic

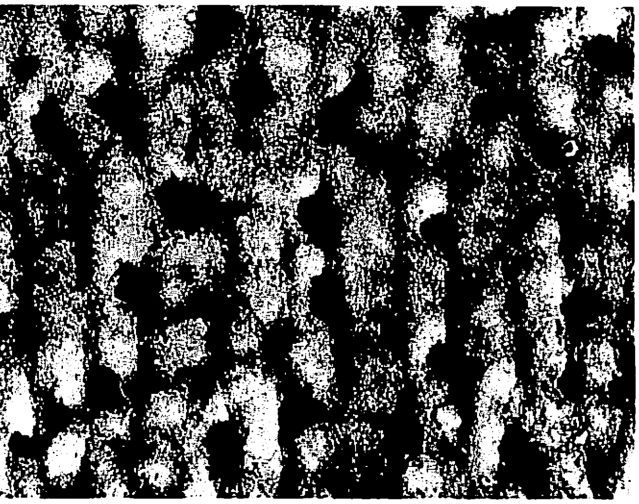


Fig. 3. Kangaroo (*Macropus sp.*), attritional facet of occlusal surface of cheek tooth treated by airpolishing: enamel tubules opening at surface are increased in size by this process. 20 kV BSE. Fieldwidth 32 µm

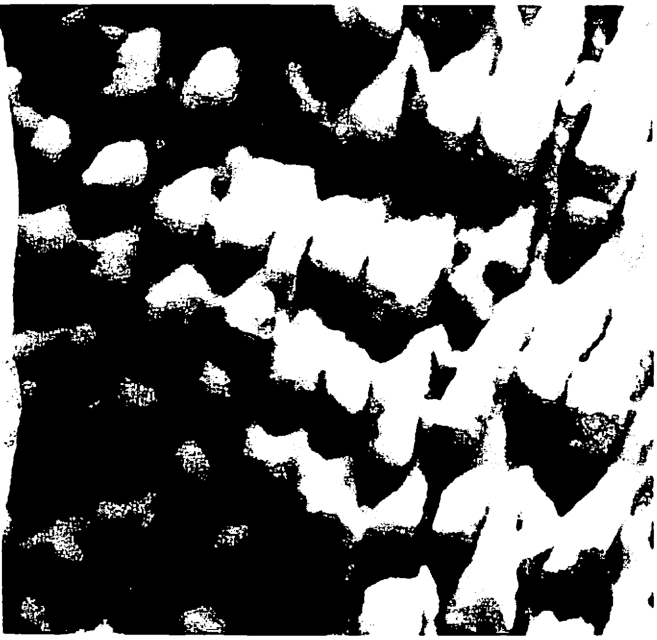


Fig. 4. Black rhinoceros (*Diceros bicornis*) enamel showing near transverse and obliquely intercepted prisms after airpolishing: the surface is most worn at prism boundary discontinuities. 20 kV CBSE. Fieldwidth 37 µm

fragments could cleave away from the tissue at the tubule wall (Fig. 3).

(C) Prisms were brought into relief due to the removal of more tissue at the prism boundary discontinuities by a microfracture mechanism paralleling the expansion of the enamel tubules. Transversely intercepted prisms thus remain as mounds with the highest point most remote from the surrounding prism boundaries (Fig. 4).

(D) At much cruder scale, zones of prisms lying more nearly parallel with a cut surface (parazones) were removed more rapidly than zones with more transversely cut prisms (diazones). It is suggested that this results from the more frequent dislodgement of long fragments of entire prisms where their boundaries are nearly parallel with the surface (Fig. 5).

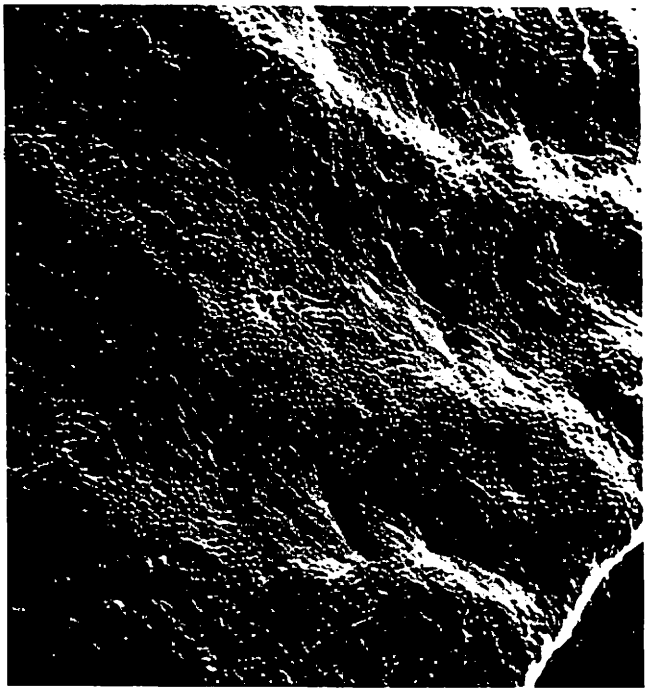


Fig. 5. Human enamel sectioned longitudinally and at 45° to surface to cut prisms zones at contrasting angles, then airpolished. Transversely cut prisms (diazones) are more wear resistant and stand proud of the surface. 10 kV SE. Fieldwidth 380 µm

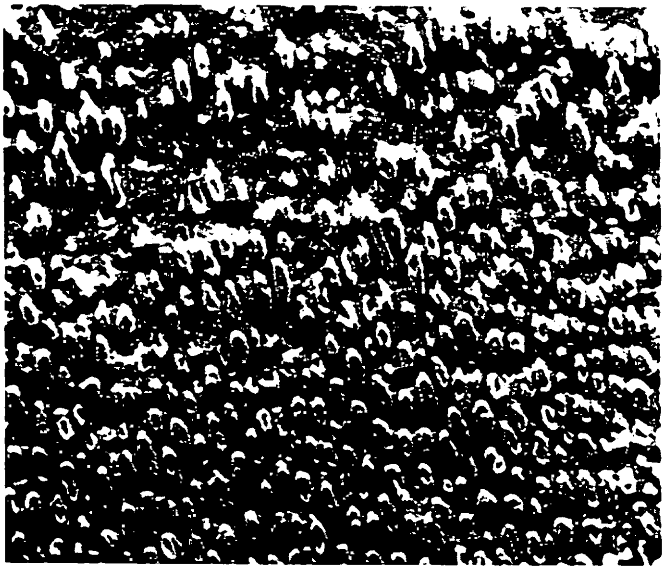


Fig. 6. Black rhinoceros (*Diceros bicornis*) dentine, oblique transverse section to tubules, airpolished showing peritubular dentine zones standing proud. 20 kV SE. Fieldwidth 377 µm

Dentine

The intertubular mineralised collagen phase in dentine was removed more rapidly than the peritubular hypermineral-

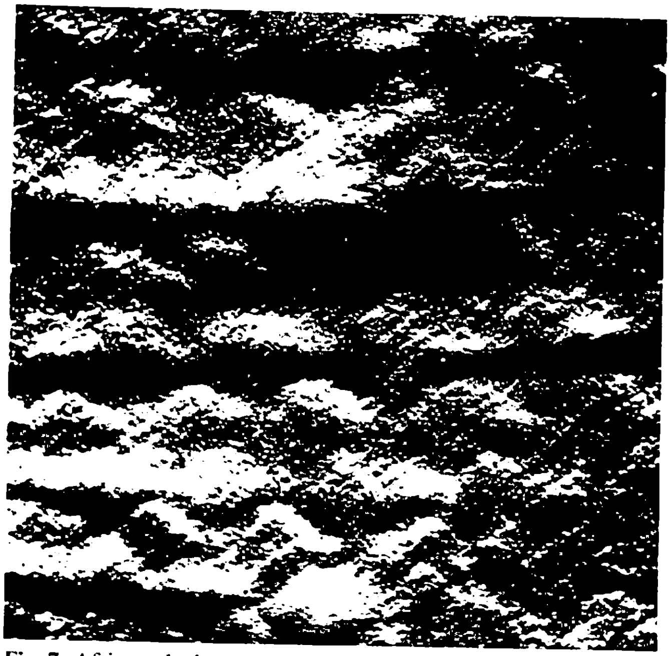


Fig. 7. African elephant (*Loxodonta africana*) ivory (TS tusk dentine) airpolished to produce surface relief related (a) to tubule orientation – the chequerboard pattern, and (b) variations in mineralisation density – the incremental lines running obliquely from bottom left to top right. 20 kV BSE-ET image. Fieldwidth 4.25 mm

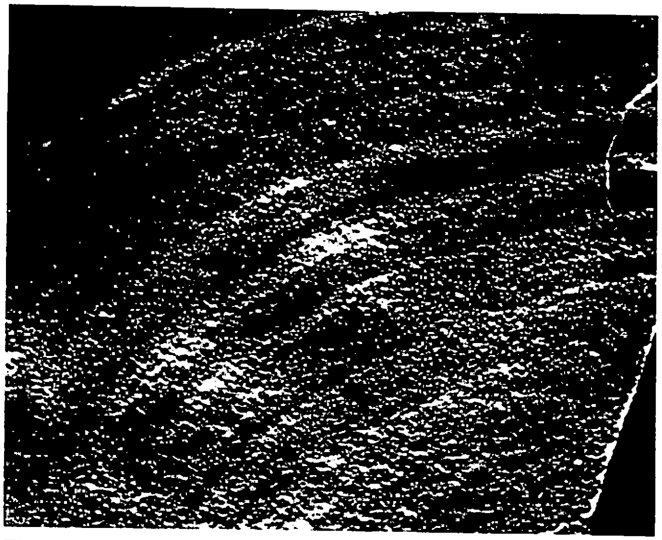


Fig. 8. Sperm whale (*Physeter catodon*) dentine, TS mandibular tooth showing etch relief of incremental lines produced by airpolishing. BSE-ET 20 kV. Fieldwidth 4 mm

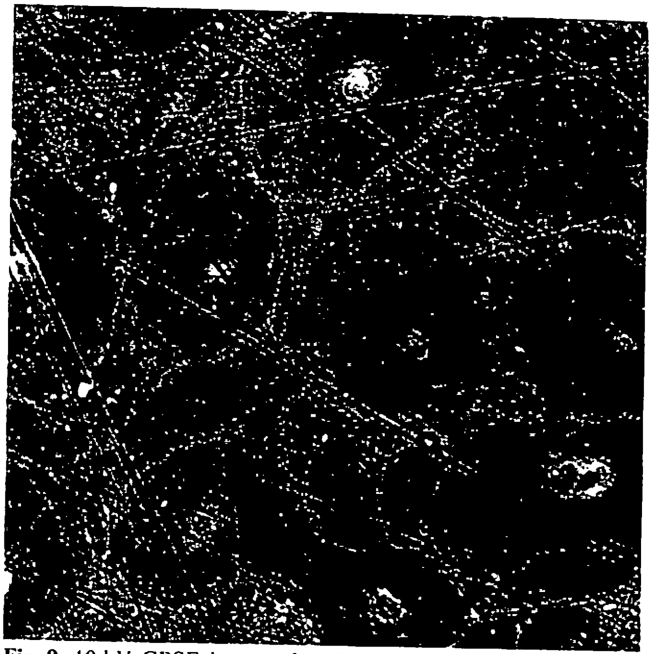


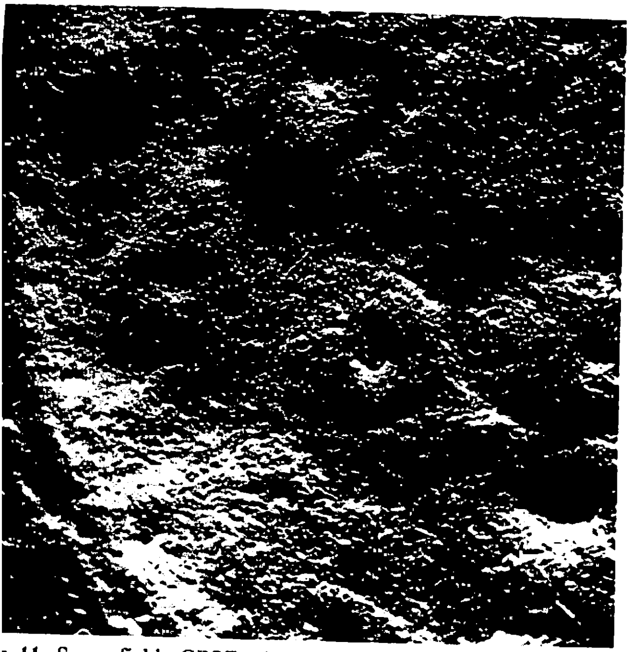
Fig. 9. 10 kV CBSE image of surface of 150 μm thick transverse section of dog tibia finished on 600 grit carborundum paper – hence scratches. Non-topographic contrast is due to density variations; whiter, interstitial lamellar areas are more densely mineralised. Fieldwidth 516 μm

Fig. 10. Same field seen in circularly polarised light: bright areas are those which have collagen lamellae more parallel with the plane of section. Field width 516 μm

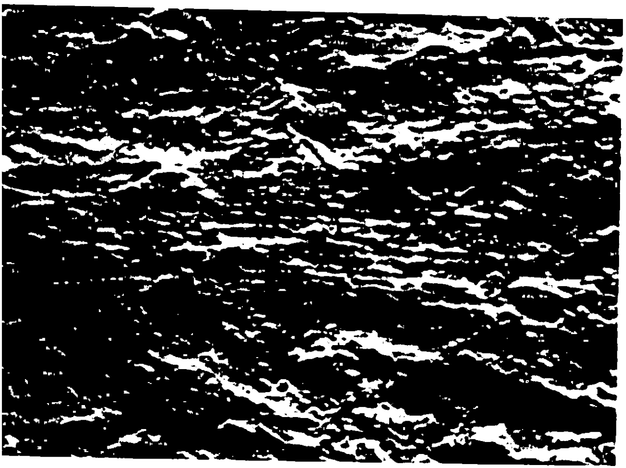
ised zones, which are particularly prominent in horse and rhinoceros dentine (Fig. 6). This presumably reflects the increased density of the peritubular zones.

At a grosser scale within dentine, there are etching effects related separately to orientation and density (Figs. 7, 8). In elephant ivory (dentine) there is a complex pattern occasioned by the contrasting orientation of adjacent columns of alternating sinusoidally oscillating dentine tubules (Miles and Boyde 1961). The resulting chequerboard pattern is brought into prominent etch relief after airpolishing (Fig. 7) showing that the rate of removal of dentine depends upon the orientation of the dentine tubules.

Sperm whale dentine is characterised by the presence of prominent incremental layering reflecting differences in the degree of mineralisation of the tissue (Boyde 1980). Under airpolishing attack, the softer, less mineralised layers



;. 11. Same field, CBSE 10 kV, after airpolishing for 10 secs, noving 70 µm of tissue, except at left, where part of original rface was protected by aperture. Fieldwidth 516 µm



. 12. Higher magnification from another field on transverse sec-
ı of dog tibia, showing etch relief related to lamellar structure.
10 kV. Fieldwidth 445 µm

removed more rapidly than the intervening, better min-
lised layers (Fig. 8). Similar incremental-line related re-
can be seen in the airpolished elephant ivory specimen
wn in Fig. 7.

le

alogous etching effects were found in bone. In transverse
ions of compact bone, different osteones and interstitial
ıellae patches were etched to varying degrees (Figs. 9–11).
ese etching effects might have reflected different degrees
ineralisation or the fact that different osteones contain
oportions of lamellae with collagen fibre bundles
nt orientations (Ascenzi and Bonucci
ect interpretation of the details
tive study was made in

which the density was determined by backscattered electron imaging (Boyde and Jones 1983), and the collagen orientation determined by circularly polarised light (CPL) (Boyde et al. 1984, in press) before the samples were airpolished. It was found that areas appearing bright in CPL, i.e. those with collagen fibres lying nearly parallel with the plane of section, were eroded more rapidly than the CPL dark areas even though these same areas appeared brighter (i.e. denser) in BSE images (Figs. 9–11). This demonstrated that, as in the case of enamel and dentine, airpolishing erosion is more rapid where structures are lying parallel with a surface under attack.

At higher magnifications it could be seen that the lamellar structure in bone also produces a localised etch relief, presumably again related to differences in the etching rate of transversely and longitudinally intercepted collagen fibre bundles (Fig. 12).

Discussion

The results of this study show that erosion due to soft-particle impact abrasion ("airpolishing") causes the development of etch relief related to both compositional and orientational changes in adjacent microscopic areas. However, differences in the orientation of tissue components seems to be more important in determining "etching" rates than density variations, except where these are gross. Further, in the case of enamel, dentine, and bone we can point to a general correlation between surface-parallelism of a particular class of structure or microstructure and an increased airpolishing erosion rate. Thus whole prisms in enamel, whole intertubular zones in dentine and domains of collagen bundles in bone appear to be able to be cleaved from a surface if parallel with the surface.

At a much finer scale it is possible to speculate that the preferential removal of enamel at transversely cut prism boundaries and marsupial enamel tubules also relates to the fact that the submicroscopic enamel crystallites can cleave from the structure because these features do create local surface which is perpendicular to the main surface under attack: the crystallites are parallel to these local extensions of the surface.

The above effects are paralleled by events which occur during polishing of hard tissue sections particularly when soft, resilient polishing substrates are employed. With "airpolishing", however, it is possible to employ these artefacts usefully in structure investigation. The orientation dependent effects in normal polishing, where the aim is to produce a relief-free surface, must be regarded as a nuisance. For example, density determination either by BSE imaging of section surfaces or microradiography of sections would be influenced by the development of polishing topography.

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