Influence of structural hierarchy on the fracture behaviour of tooth enamel

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Tooth enamel has the critical role of enabling the mastication of food and also of protecting the underlying vital dentin and pulp structure. Unlike most vital tissue, enamel has no ability to repair or remodel and as such has had to develop robust damage tolerance to withstand contact fatigue events throughout the lifetime of a species. To achieve such behaviour, enamel has evolved a complex hierarchical structure that varies slightly between different species. The major component of enamel is apatite in the form of crystallite fibres with a nanometre-sized diameter that extend from the dentin–enamel junction to the oral surface. These crystallites are bound together by proteins and peptides into a range of hierarchical structures from micrometre diameter prisms to 50–100 µm diameter bundles of prisms known as Hunter–Schreger bands. As a consequence of such complex structural organization, the damage tolerance of enamel increases through various toughening mechanisms in the hierarchy but at the expense of fracture strength. This review critically evaluates the role of hierarchy on the development of the R-curve and the stress–strain behaviour. It attempts to identify and quantify the multiple mechanisms responsible for this behaviour as well as their impact on damage tolerance.

1. Introduction

Enamel is the most highly mineralized tissue (up to 96% by volume [1]), and thus the hardest material in the...
human body, and is located in the outermost layer of the crown of a tooth. Its biological function is to protect the underlying soft dentin and pulp from mechanical forces and chemical attacks during tooth function. Enamel is not a living tissue and thus its loss owing to abrasion and fracture is irrecoverable. This tissue has frequently been described as a brittle material in earlier studies [2–4] and thus relatively little attention has been given to its structure–fracture behaviour relations in comparison with those of bone and dentin. On the other hand, it was revealed that, in spite of pre-existence of cracks in the outer surface [5], enamel can survive millions of chewing cycles with mastication forces ranging between 28 and 1200 N [6]. This raised the question of how an inherently brittle tissue sustains cracks without catastrophic fracture [5]. Identifying the features that enable this performance in biological materials with the aim of implementing them to improve engineering materials has become an increasingly important area over many years. This has resulted in renewed interest with further enamel studies employing advanced fracture mechanics being published [7–13]. These authors reported that bulk enamel exhibits increasing fracture toughness with crack extension (R-curve behaviour) compared with brittle materials that generally have a single value of fracture toughness. This behaviour of enamel is attributed to the interaction of cracks with its hierarchical microstructure, which will be classified in this review according to Koenigswald and Clemen’s structural description [14,15]. The basic structural element in enamel is a single hydroxyapatite (HAP) crystallite (level 0). Bundles of these crystallites (level 1) form the micro-scale building blocks known as prisms (p) and the interprismatic matrix (ipm; level 2; the term ‘prism’ is used synonymously with ‘rod’). The arrangement and orientation of prisms determines the next level of hierarchy termed as enamel types (level 3), which can be broadly classified as radial and decussating enamel. The next level of hierarchy (level 4) is called the ‘schmelzmuster’ (enamel pattern), which defines the three-dimensional distribution of the different enamel types in a tooth. As a typical representation, an overview of the hierarchical organization in bovine enamel is illustrated in figure 1. In addition, enamel is known to contain a limited amount of soft organic matter (protein/peptides, firmly
Hierarchical mechanics model (image from Bechtle et al. [16]) and supporting experimental results: quasi-self-similar brick and mortar hierarchical model loaded in tension according to Gao and co-workers [28] (a). The brick represents hard particles (black), which are enveloped by mortar, the soft phase (grey), at each level. The composite structure at level $n$ forms the hard particles at level $(n + 1)$. One basic assumption of this model is that hard particles carry the entire tensile load. The soft phase transfers the tensile load via shear to the particles. The reduction in strength (b) and elastic modulus (c) in bovine enamel with increasing hierarchy was experimentally assessed by Bechtle et al. [16] and applied to verify the model proposed by Gao and co-workers [28]. The orange, green and blue regions represent, respectively, the experimental data, properties of basic constituents and calculations using Gao’s model.

The combination of being hard, stiff and tough—as observed generally in mineralized biological tissues—is a desired mechanical property also for engineering materials and hence much recent effort has been directed at exploring the basic mechanical principles associated with these hierarchical microstructures both experimentally and theoretically. Novel experimental techniques have enabled researchers to assess the individual response of microstructures over different length scales and constituent materials by combining nano- to macro-scale mechanical tests together with high-resolution microscopes and synchrotron X-ray diffraction. A considerable amount of literature has been published on bone identifying its hierarchical deformation mechanisms, as summarized by Gupta & Zioupos [27]. This helped form the basis for the hierarchical mechanics model developed by Gao and co-workers [28] based on suggestions from...
Jäger & Fratzl [29]. Furthermore, by applying fracture mechanics concepts to a two-dimensional, quasi-self-similar structure resembling bone, they derived formulae for mechanical properties, including strength and elastic modulus for higher structural levels as functions of the material properties of subordinate hierarchical levels (e.g. protein content, mineral particle size or particle strength). The reader is referred to recent publications [28–33] for more details.

The basic concept of the model is visualized in figure 2a. The quasi-self-similar structure is considered to be built of hard mineral particles enveloped by a soft protein matrix at the lowest hierarchical level. This composite structure at level \( n \) further forms hard particles at level \((n + 1)\) which again are enveloped by soft matrix. This means that each additional level of hierarchy adds more protein to the composite structure (assuming that the mineral volume fraction of each hierarchy is constant), which improves damage tolerance at the expense of the elastic modulus and strength under tension as experimentally observed in enamel from level 1 to level 2 by Bechtle et al. [16] (figure 2b,c). Bechtle et al. analysed these experimental results with Gao’s model and they were found to be in good agreement (figure 2b,c).

Besides measurements of stress–strain curves at different length scales, there have been contributions in enamel research investigating the submicrometre fracture toughness and toughening mechanisms with small-scale testing methods [25,26]. Apart from these, additional R-curve measurements provided greater insight into the orientation-related factors associated with crack growth resistance of enamel at larger scales. The objective of this review is to bring together the findings reported in different studies associated with different hierarchical levels so as to provide a deeper understanding of the role of hierarchy in the fracture behaviour of enamel. This requires a detailed description of its microstructural features. Thus, we initially summarize the structural features at individual hierarchical levels, and then analyse the role and contribution of the corresponding features to the fracture behaviour (focusing on fracture toughness, fracture strength, deformation curves, toughening mechanisms). An overview of the characterization methods used in the literature [2–13,16,25,26,34–36] that are reviewed here for assessing the fracture behaviour of enamel at different length scales can be found in the electronic supplementary material. Finally, we briefly summarize the findings.

2. Structural characteristics of enamel at different hierarchical levels and their role on fracture behaviour

(a) Hierarchical level 0: single crystallite

(i) Structural features

The hierarchical level 0 of enamel is represented by a single crystallite (figure 1). The reported values of the cross-sectional dimensions of the mature crystallites from various sources were listed by Daculsi & Kerebel [37], and they ranged between 15 and 50 nm in thickness, and between 40 and 150 nm in width. The length of the crystallites was shown to be at least 100 \( \mu \)m [38]; however, they are believed to extend without interruption from the dentin–enamel junction (DEJ) to the outer enamel surface (OES) [39]. It should also be mentioned here that the crystallites of enamel are a carbonated form of HAP that differs slightly from synthetic HAP [39]. Additionally, HAP crystallites in enamel are derived from a biomineralization process which results in imperfections as evidenced by high-resolution electron microscopy [37,40]. Enamel is formed by ameloblast cells which secrete the matrix proteins that are responsible for deposition of the mineral crystallites [39]. Matrix proteins self-assemble to form nanospheres surrounding the crystallites and force them to grow along their long axis and inhibit crystal–crystal fusion. During maturation, proteins break down and are removed from the matrix to create space, so that the pre-existing crystallites can grow in width and thickness. This allows the crystallites to come into contact and may induce mechanical stresses distorting the atomic arrangement and forming dislocations. Moreover, the external surfaces of the crystallites are not smooth but display frequent step-like irregularities.
The influence of the dimensions and imperfections of the enamel crystallites is analysed in the following.

(ii) Implications for fracture behaviour
The specific nanometre-sized scale of the enamel HAP crystallites has evolved by nature as postulated by Gao et al. [30–33] in order to ensure optimum fracture strength of the mineral particles. Brittle materials fail by stress concentrations that arise owing to the presence of crack-like flaws before reaching their theoretical strength \( \sigma_{\text{th}} \), which is the tensile stress necessary to break primary chemical bonds in a defect-free material. According to Griffith’s theory, fracture stress depends on the material parameters elastic modulus \( E \) and surface energy \( \gamma \) and on the crack size \( a \). If the dimension of the biominal crystallites drops below a critical size, they become defect insensitive and the atomic bonds control the strength [30]. The critical length scale for defect tolerance, \( a^* \), is estimated as

\[
a^* \approx \alpha \frac{\gamma E}{\sigma_{\text{th}}^2},
\]

where \( \alpha \) is a parameter dependent upon crack geometry and is approximately equal to \( \sqrt{\pi} \) for a half-cracked platelet [30]. By taking the parameters \( \gamma_{\text{HAP}} = 1 \text{ J m}^{-2} \) [42], \( E_{\text{HAP}} = 125 \text{ GPa} \) [43] and \( \sigma_{\text{th}} = E/30 \) [30], \( a^* \) is estimated to be approximately 36 nm. This is in agreement with the dimensions of the HAP crystallites in enamel and favours the concept of the optimization of the fracture strength of the mineral crystallites and their insensitivity to minor defects. This has not been validated owing to the lack of experimental techniques enabling the assessment of the fracture strength of a single HAP crystallite isolated from a biological tissue. However, Bechtle et al. [16] calculated the fracture strength of a single enamel crystallite to be approximately 2 GPa indirectly, by combining the experimental results and analytical solutions for hierarchical materials. This value is approximately one order of magnitude higher than the strength obtained from bulk HAP (approx. 100 MPa [44]; see the electronic supplementary material, table S1) and is of the order of theoretical strength estimation of approximately 4 GPa (\( \sigma_{\text{th}} = E/30 \)). The slightly lower experimental value compared with the theoretical value may be explained by the fact that the elastic modulus of a single enamel crystallite is not reported in the literature owing to the lack of sample preparation and testing technique. Thus, the elastic modulus of an artificial micro-sized HAP single crystal (125 GPa) assessed by nanoindentation [43] is used in the calculation of the theoretical strength. Note that indentation approaches quantify material properties under multi-axial stress state (largely compressive stresses) that can differ from the properties assessed under uniaxial tension. Considering the existence of the aforementioned imperfections in the enamel crystallites, the tensile elastic modulus is expected to be lower in reality than the elastic modulus value given here. This could be the reason for the discrepancy between the estimated theoretical strength here and the reported fracture strength by Bechtle et al. [16].

(b) Hierarchical level 1: multiple crystallites

(i) Structural features
The hierarchical level 1 of enamel is associated with bundles of crystallites held together through the composite nature of enamel and imperfections in the crystallite shapes and arrangements, which will be elucidated in this section. Enamel is formed in two stages as explained above. During the initial stage, the crystallites are thin and ribbon-like structures whose unit cell is hexagonal (6/m). However, the hexagonal outline of the crystallites is lost as they press against each other during the final phase of their growth and become irregular in shape [37,39,45]. These interlocking shapes grow to fill all the available space between the crystallites [46] and hold them together (figure 3a). In addition, some other structural abnormalities such as crystallite fusion and lateral branches (bifurcations) are also evident in enamel crystallites [37,45]. Examining longitudinal sections of mature enamel reveals that HAP crystallites do not align perfectly parallel
to each other; they seem rather entangled (figure 3b,c). This imperfect arrangement may interlock the crystallites, enabling them to remain attached structurally. However, a more common view is that the crystallites are bound together by intercrystallite proteins, which are not incorporated within the crystalline HAP. It is observed that there exist remnant proteins and water in mature enamel as assessed by vibrational spectroscopy (infrared and Raman) and thermogravimetric analysis. These soft substances are postulated to fill the free space where the mineral density is low owing to imperfect crystal packing. These regions, which are 1–2.5 nm electron-lucent spaces between the crystallites, are detected frequently in transmission electron microscopic (TEM) studies [41,45]. However, it is also observed that the crystallites appear to be in direct contact with each other. This indicates that there may be protein layers between crystallites but they are not homogeneously distributed. The type of intercrystallite proteins in mature enamel was reported as amelogenin, which has a hydrophobic character, the significance of which will be described below [47,48].

(ii) Implications for fracture behaviour

Characterization of the mechanical properties of the constituents at level 1 solely, without any contribution from higher-order structural motifs and materials, has only a recent history. The first study on the small-scale mechanical characterization of enamel was published by Chan et al. [49] using a micro-cantilever bending technique. Following this study, several others used the same methodology to investigate the properties of isolated crystallites from different tooth types [16,50]. The resultant mechanical properties from the bundles of crystallites l (loaded parallel to the crystallite long axis) from various studies are listed in the electronic supplementary material, table S1. The micrographs of the samples before and after loading together with the resultant stress–strain curves are illustrated in figure 4a,b. Chan et al. [50] investigated the influence of dehydration owing to focused ion beam (FIB) treatment and could not find a significant effect. Although there is substantial scatter in the failure strengths obtained from different studies (that can be attributed to different mineral content of the tested samples), the fracture strength of this
level is approximately 1 GPa. This is still one order of magnitude higher than bulk HAP strength (approx. 100 MPa [44]) but two times lower than the strength of a single HAP crystallite (approx. 2 GPa [16]). This difference can be explained by the composite nature of the first hierarchical level, as proteins are also incorporated. Moreover, there is a higher probability of the presence of defects and imperfections in a bundle of crystallites. The elastic modulus values measured via micro-cantilever bending experiments are significantly lower than the elastic modulus of bulk HAP (approx. 80 GPa [44]) assessed under bending. The possibility of degradation in the HAP crystallites owing to FIB treatment was elaborated using TEM [51,52], where HAP is reported to be structurally resistant to FIB-induced damage. Moreover, Chan et al. [49] revealed no significant difference in the elastic modulus and hardness of enamel before and after FIB preparation, which would not be expected in the presence of protein degradation [53]. Consequently, it can be assumed that FIB treatment does not have a significant effect on the measured mechanical properties of enamel. The resulting low elastic modulus values were attributed to the dissolution of minerals in the micro-cantilevers by the acid treatment (this etching procedure is necessary to make the prism boundaries visible and locate the milling site correctly) [49]. Moreover, as mentioned earlier, the HAP crystallites differ slightly from synthetic HAP in chemistry and structure, which may also alter their mechanical properties [35].

In soft-matrix-based structural composites, progressive failure behaviour (saw-tooth-shaped stress–strain curve) is expected to occur, where the reinforcing fibres fail in sequence beginning with the weakest [54,55]. As each succeeding fibre fails, the remaining intact fibres share the increased load released by the broken fibres that is distributed by the matrix [55]. However, the commonly observed fracture behaviour at this hierarchical level was catastrophic failure after a near entirely linear–elastic deformation as shown in figure 4b. There is a hint of slight nonlinear behaviour from beyond two-thirds of the maximum stress, but whether this is associated with progressive crystallite failure or shear between the crystallites is unclear. Alternatively, the catastrophic failure indicates that all the HAP crystallites in the micro-cantilever bending test failed simultaneously. This process may be attributed to the lack of load transfer ability of the intercrystallite proteins owing to their limited content (less than 2 vol.%) compared with the matrix content in the above-mentioned artificial composites (greater than 30 vol.% [55]). The fracture toughness in this orientation, where the crystallites are parallel to the tensile stress direction (crystallite fracture mode), was assessed by Bechtle et al. [26] by using notched micro-cantilevers as schematically illustrated in the electronic supplementary material, figure S1. The notch placed with an off-set from the cantilever’s fixed ends introduced a shear component $K_I$ at the crack tip in addition to the tensile component $K_{II}$ [26]. $K_I$ and $K_{II}$ acted simultaneously at the crack tip when fracture occurred but the latter was found to be very small (see the electronic supplementary material, table S2). Upon computing the corresponding geometry function for the triangular notched sample geometry, Bechtle et al. [26] assessed the $K_{IC}$ values as 0.44–0.91 MPa m$^{0.5}$, which lies in the range of synthetic HAP values, indicating the minor role of intercrystallite proteins in fracture toughness in this orientation. However, when the crack propagation is induced between the crystallites, the fracture toughness values of bovine enamel showed a much larger scatter [25]. Ang et al. [25] measured the crack opening displacement at the crack tip region induced by Vickers indentation and evaluated both mode I $K_I$ and mode III $K_{III}$ crack tip toughness (which is the toughness at crack initiation, where no extrinsic toughening mechanisms are yet activated) by applying the cohesive zone solution of the Dugdale–Muskhelishvili crack model and Irwin’s ‘near-field’ solution. The cracks propagated mainly along the prism boundaries (considered as hierarchical level 2 in this review) and within the prisms, the latter corresponding to hierarchical level 1. The values of crack tip toughness $K_I$ and $K_{III}$ ranged between 0.52 and 1.62 MPa m$^{0.5}$ and between 0.05 and 0.15 MPa m$^{0.5}$, respectively [25]. The authors also computed the crack tip closure stresses as 434–779 MPa for a cohesive zone width of 24–36 nm and a length of 1.6–3.2 µm, which may be attributed to microcracking, bridging by protein filaments and crystallite ligaments [25]. The last are the toughening mechanisms prevailing at the first hierarchical level of enamel as illustrated in figure 5. Microcracks of 100–500 nm in length were observed around the main crack within an
enamel prism and followed the contours of the crystallites (figure 5a). These microcracks arise as the main crack is obstructed by the frictional mineral–mineral interactions associated with the irregular cross-sectional shapes of the crystallites. The latter behaviour was observed by Ang et al. [25] when they examined the crack profiles using atomic force microscopy (AFM) and identified nano-sized approximately 85 nm frictional bridges upon scanning close to the crack tip as shown in figure 5c. In addition, unbroken crystallite ligaments bridging the main crack propagating across the long axis of the crystallites are demonstrated in figure 5a. The entangling arrangement of the crystallites together with the bifurcations and fusions within them complicates the crack propagation along the crystallite axis by the formation of crystallite bridges as can be seen in a TEM cross section (figure 5b) showing a crack induced by nanoindentation forming around the long axis of the crystallites [56]. Aside from the observations of microcracks and crystallite bridges, both the studies identified the presence of protein ligament bridges when the crack propagates between crystallites.

Figure 4. Micro-cantilever bending tests. (Images from Bechtle et al. [16].) (a) Triangular cross-section micrometre-sized (<5 μm in width and height, approx. 15 μm in length) FIB-milled cantilevers within the prisms avoiding the prism sheaths. These samples consisted of HAP crystallites only, representing the hierarchical level 1. Loading was achieved by using the optical microscope of the nanoindentation system, and the Berkovich tip was positioned at the cantilever’s free end, as marked by the arrow. A typical stress–strain curve of HAP crystallites loaded parallel to their long axis is displayed in (b). The inset in (b) displays the post-failure morphology, where the broken crystallites protrude from the fracture surface. (c) For characterization of the second hierarchical level in enamel, larger cantilevers (10–15 μm in width and height, 40–50 μm in length) covering several prisms were prepared and tested with the same method. A representative stress–strain curve for the multiple prisms is demonstrated in (d). Note the increased nonlinearity in the stress–strain curve compared with level 1 that is attributed to the increased protein content with increasing hierarchical levels [16]. The fracture mode of the multiple prisms is shown in the inset (d), revealing the crystallite fracture within the prisms together with the crack deflection at prism boundaries that run parallel to the length of the beam. (Online version in colour.)
(c) Hierarchical level 2: multiple prisms

(i) Structural features

Enamel microstructure is a result of millions of years of evolution and it has diversified among different species and to a lesser extent between the teeth of an individual. Evolutionary development of enamel has an adaptive relationship to the stresses generated during the functioning of the tooth and is closely related to the physiological task of the dentition and the properties of the food items [57]. The enamel of lower vertebrates has the simplest structure known as ‘prismless enamel (parallel crystallite enamel)’, representing the most primitive enamel type with only one level of hierarchy (multiple crystallites). Prismless enamel is observed in the teeth of species (mainly reptiles) whose physiological task is usually limited to the capturing, piercing and holding of the prey with no contact between the opposing jaws [58,59]. The change in feeding habits to mastication and chewing resulting in higher stresses generated in teeth was accompanied by a modification of enamel microstructure from prismless to prismatic enamel in mammals [15,58]. Prismatic enamel evolved from prismless enamel as a consequence of the differentiation in the enamel formation (ameloblast) cells [58], which promoted the creation of gap regions between the bundles of crystallites forming enamel prism (rods; figure 6). Each ameloblast cell is responsible for the formation of a single prism (p) and also some ipm component, which is also associated with the hierarchical level 2. Enamel prisms are continuous from the DEJ close to the OES and have roughly cylindrical geometry but their exact shape (round, angled, arc-shaped, keyhole-shaped in cross section) may vary taxonomically and/or locally in a single tooth. Roughly, the thickness/width/diameter (depending on the shape) of prisms ranges from 1 to
7 µm, and the thickness of the ipm can vary between 0.5 and 3 µm depending on the enamel type. Crystallites within a prism are oriented roughly parallel to the prism long axis; however, in some species (such as the keyhole-shaped prisms in humans, as seen in figure 6c), they fan out from the centre towards the edges [15]. Crystallites within the ipm are parallel to one another but at an angle to the prism direction in general, whose variations will be addressed in the explanation of prism patterns.

Prisms have a borderline called a ‘prism boundary’ that is a gap zone, whose thickness appears to be 0.1–1 µm in the micrographs shown in figure 6. However, this can be misleading, because those samples were highly acid etched prior to microscopic inspection to make the boundaries visible. In the TEM micrographs of unetched samples, the prism boundaries are less prominent and are revealed as discontinuous regions with small pores (less than 50 nm) partially bridged by crystallites [56,60]. Prism boundaries have been proposed to be made up of a protein sheath [39], because these are the gap regions where the majority of the remnant proteins should be constricted during maturation. However, it has been almost impossible to observe the protein sheaths directly by structural analysis owing to the degradation of proteins by harsh sample preparation procedures. To date, there is only one report [61] demonstrating the protein sheaths (see the electronic supplementary material, figure S2). This study enabled researchers for the first time to identify the nature of enamel proteins as a three-dimensional fibrous mesh-like structure. The interprismatic proteins in mature enamel were reported to be ameloblastin and are hydrophilic [62]. The hydrophobic nature of amelogenin versus the hydrophilic nature of ameloblastin enables aqueous solutions to penetrate between the prisms but less so between the crystallites. For human enamel, this has a significant influence on the effects of bleaching agents penetrating teeth [63].

The appearance of prism boundaries and differences in prism–ipm packing (or the way the prisms and ipm are arranged relative to another) designated as prism patterns are the most striking characteristics at this level that vary among mammalian taxa [64]. The prisms may have ‘closed’ boundaries, surrounding the prisms completely or ‘open’ (incomplete) boundaries, where the ipm anastomoses to prisms and forms a single continuum as shown in figure 6. Many species show a combination of both [15]. The arrangement of prisms and ipm can be simply distinguished between honeycomb and sandwich patterns (figure 7). In the honeycomb pattern, the ipm surrounds the prisms that are organized roughly in a disordered way. In the primitive condition, the orientation of the crystallites in the ipm remains more or less parallel with that of the crystallites of prisms, which has however diverged with time and the intersecting angle between the crystallites of the ipm and prisms has increased [65]. In the sandwich pattern, prisms are arranged in approximately vertical rows separated by the ipm layers (in these arrangements the ipm is also called ‘inter-row sheets’). The ipm layers have bifurcations that create imperfections in the arrays as shown in figure 7a. The crystallites of the prisms and ipm intersect at almost right angles in the sandwich pattern. This is also known as ‘crystallite decussation’ and is believed to emerge at a more derivative stage of enamel evolution as a fracture-resisting mechanism at this hierarchical level [65].
Figure 7. Sandwich pattern of prism rows and ipm layers (inter-row sheets; a); honeycomb pattern of prisms surrounded by ipm (b) in bovine enamel. Bifurcations in the ipm layers are marked with the arrows. (Online version in colour.)

(ii) Implications for fracture behaviour

The fracture strength and elastic modulus of several prisms isolated from the bulk enamel (figure 4c,d) were reported to be 478 ± 93 MPa and 36 ± 8 GPa, respectively [16]. There was almost a 50% decrease in the fracture strength and elastic modulus values compared with level 1, which was attributed to the possibly higher protein content of level 2 by Bechtle et al. according to Gao’s hierarchical model, as introduced previously [16]. Gao calculated the overall mineral content, \( \varphi_{\text{total}} \), of hierarchical materials with the following relationship:

\[
\varphi_{\text{total}} = \varphi_1 \varphi_2 \ldots \varphi_n, \tag{2.2}
\]

where \( \varphi_n \) is the mineral volume fraction of the composite structure at level \( n \) [31]. Assuming the mineral volume fraction of each hierarchical level is constant (\( \varphi_1 = \varphi_2 = \varphi_n \)), the mineral volume fraction decreases with increasing hierarchical structuring. In addition, the nonlinear character of the stress–strain curves appears to have increased from level 1 to level 2 (figure 4d). The subtle chemical differences between the interprismatic proteins and the intercrystallite proteins were previously mentioned. The former are hydrophilic, whereas the latter are hydrophobic. Consequently, the increased nonlinearity at level 2 could be related to the higher deformability of the more porous water-filled protein structure at the prism boundaries compared with the limited water present within the prisms. Furthermore, the crystallite fracture within the prisms is accompanied by crack deflection at the prism boundaries, indicating also the change in fracture mode at this level. The \( K_{IC} \) results assessed by FIB-notched micro-cantilever bending were 0.83–0.89 MPa m\(^{0.5}\) [26]. The mode I crack tip fracture toughness values estimated by Irwin’s crack tip solution for the second hierarchical level of enamel are 0.64–0.94 MPa m\(^{0.5}\) for the cracks propagating along the prism boundaries [25]. Results from the earlier Vickers indentation studies on human enamel reporting the fracture toughness are also associated here with the hierarchical level 2 owing to the corresponding crack lengths (approx. 50 \( \mu \)m). These values ranged from 0.68 to 1.3 MPa m\(^{0.5}\). Correspondingly, crack closure stresses near the crack tip along the prism boundaries were estimated as 168–754 MPa for a cohesive zone length of 10–1.6 \( \mu \)m [25].

Evolution of prismatic enamel from prismless enamel has resulted in the formation of prism boundaries that introduced a weak path for crack propagation. This serves in the first place to localize the cracks into confined zones, which control the crack path direction. This guided crack growth, resulting from various crack-arresting mechanisms, contains the crack within the tooth and so prevents chipping of enamel [8]. These mechanisms associated with level 2 are represented in figure 8. Note that some of these mechanisms are detected only in specific parts of the tissue, which will be addressed at level 4 of the schmelzmuster. It was previously mentioned that the high angle (approx. 90°) between the prisms and ipm sheets (sandwich pattern) creates a prism–ipm (crystallite) decussation, which differs from the decussation of prism bundles (HSBs). This
is commonly observed in ungulates and here the crack deflection occurs at right angles owing to prism–ipm decussation evident in bovine enamel as presented in figure 8a. Such deflections result in a decrease in the local stress intensity at the crack tip [7]. In addition, some of the ipm sheets and the prisms have to be broken for the crack to advance, manifesting the reinforcing strategy of enamel at this level of hierarchy. Uncracked ligament bridging of proteins residing between the prisms is evident from observations of the crack path in both human and bovine enamel as an energy-dissipating mechanism through extension/unfolding of the peptide chains [7–9] and is illustrated in figure 8d. Open prism boundaries where the ipm anastomoses to prisms at the tail region forming a single continuum were also introduced previously in the structural features section. These crystallite interconnections between the prisms and ipm are responsible for crack bridges (also evident in [7,25]) when they run along the prism–ipm boundaries as shown in figure 8e. Unbroken prism ligaments (a few prisms), as demonstrated in figure 8f, are also reported in various studies of human and bovine enamel and have a significant contribution to the fracture toughness by shielding the crack tip and further reducing the local stress intensity available to drive fracture [7,8,11,12,25]. Observations of the crack paths in transversal oriented samples revealed crack branching (figure 8b) as a major visible toughening mechanism [8,11,12], whereas the term ‘meandering cracks’ was introduced [11] to describe branches that rejoin the main crack again after some micrometres of propagation. Crack branching yields an increase in the crack surface with increased energy consumption, which is otherwise available for crack advance [66]. Finally, microcracking about the main crack is detected in enamel [7,9,12,16,25] that is located in the prism boundaries and ipm as demonstrated in figure 8c. The contribution of microcracking in the vicinity of a crack tip creates dilatation of the structure and as a result reduces the effective elastic modulus in the process zone, thereby lowering the crack tip stresses and the effective stress intensity [7,66]. In terms of crack growth, microcracks contributing to the total crack surface are generated which consequently increases the energy required [66].
(d) Hierarchical level 3: enamel types

(i) Structural features

The enamel types in prismatic enamel are defined by whether the prisms are parallel to each other (radial enamel) or cross each other with different orientations (decussating enamel), as demonstrated in figure 1. Radial enamel is characterized by prisms developing radially in accordance to the DEJ and is considered the most basic prismatic enamel type. Modified radial enamel and additional enamel types have developed over time to meet specific biomechanical demands related to change in feeding habits and body size that gave rise to higher and multidirectional stresses acting on enamel [57,65]. The derived enamel types such as decussating enamel did not replace radial enamel in many species but arose in combination with it, forming a complex schmelzmuster [65]. There is a huge variety in prism decussation patterns among different species, but the most commonly observed specific mode is HSBs, where prisms decussate in alternating layers. HSBs are described by the inclination and thickness of the bands. The inclination of the bands can be readily seen in vertical sections of teeth and they are found to be primitively perpendicular to the DEJ, and inclined upwards to the OES in more advanced states. The thickness of the bands is usually in the range of 10–20 prisms. Regular bifurcation of the HSBs is reported by Koenigswald et al. [67] and regarded as another important feature of the bands.

(ii) Implications for fracture behaviour

The stress–strain behaviour cannot be analysed for level 3 in this review as isolated characterization of radial enamel and HSBs has not yet been published. Their dimensions (approx. 200–600 \( \mu \)m in cross section) complicate both the macro- and micro-scale sample preparation. However, preparation of compact tension (CT) specimens was possible for R-curve tests. Therefore, only the fracture toughness and toughening mechanisms associated with radial enamel of parallel aligned prisms and decussating enamel (HSBs with periodically decussating prism bundles) can be compared. The fracture toughness of isolated radial and decussating enamel were quantified by Yahyazadehfar et al. [12] using incremental crack growth measurements with inset CT specimens of human enamel and the results revealed that both types undergo an increase in crack growth resistance; in other words, they exhibit rising R-curve behaviour. Both the initiation toughness \( K_\sigma \) and the maximum toughness \( K_c \) (the magnitude of stress intensity at the onset of unstable fracture) are found to be substantially higher in decussating enamel (see the electronic supplementary material, table S2), which can be attributed to the potency of the toughening mechanisms prevailing in different enamel types [12].

The representative crack growth paths in the radial and decussating enamel resulting from the aforementioned study are presented in the electronic supplementary material. The most prominent toughening mechanism in radial enamel was identified to be crack branching, which often served as a precursor to the generation of unbroken ligaments of prism bundles each comprising a relatively limited number of prisms (5–10) [12]. However, within decussating enamel, crack bridging was more dominant than crack branching and consisted of larger unbroken prism bundles that comprised between 10 and 50 individual prisms [12]. More details regarding the superiority of enamel with prism decussation over radial enamel in stopping crack propagation are considered in the electronic supplementary material.

(e) Hierarchical level 4: schmelzmuster

(i) Structural features

The schmelzmuster describes the spatial distribution of different enamel types in a tooth according to the hierarchical terminology formulated by Koenigswald & Clemens [14]. As noted in the Introduction, various enamel types exist in a single tooth. For example, in most mammals,
schmelzmuster consists of radial enamel (parallel arranged prisms) in the outer and decussation enamel (crossing groups of prisms) in the inner enamel [15] (figure 1). The consequences of this configuration are elaborated later in §2e(ii). The change in the enamel types causes some other compositional and structural alterations from outer to inner enamel. For instance, the prism–ipm arrangement changes from a honeycomb pattern in the outer enamel to a sandwich structure in the inner enamel (specific to bovine enamel). Additionally, enamel prisms do not simply run straight from the DEJ towards the outer surface but they locally bend, twist and change their position [68]. In human enamel, as a function of the ameloblast cell movements, strongly undulating prisms in the inner enamel are observed (this is less pronounced in the inner region of bovine enamel), whereas prisms follow a straight course in the cervical (innermost) and outer regions of enamel [68]. Finally, it is well documented that the protein content of enamel increases from the outer to inner region as verified by He et al. [6] based upon Raman spectroscopic analysis.

(ii) Implications for fracture behaviour

The role of the spatial distribution of different enamel types (with regards to the corresponding structural and compositional variations from outer to inner enamel) in the fracture behaviour of the tissue is elaborated in this section. For this purpose, stress–strain and crack growth resistance measurements performed on bulk enamel samples, which represent the hierarchical level 4 (and containing all the subordinate levels), are presented. To the best of the authors’ knowledge, there exists only a single study reporting the strength of bulk enamel as 11–42 MPa with a tensile testing set-up [36] (as illustrated in the electronic supplementary material, figure S1). The limitations of these studies are that the deformation and resultant stress–strain curves were not measured. Only a recent dissertation provided greater insight into the deformation and fracture behaviour of enamel using flexure specimens from bovine incisors owing to their larger size and thicker enamel layer [9]. Bending experiments were performed using a custom-made three-point bending device with two sample groups designated as transversal and longitudinal. The orientations of the prisms within each sample group are illustrated by the schematic drawings in figure 9a.

In the case of transversal samples, the prisms were oriented perpendicular to the long axis of the sample and both the outer and inner enamel located on the tensile side of the specimen. The longitudinal samples were tested in such a direction that cracks grew from the outer to the inner enamel. The mean strength, elastic modulus and strain to failure values of six samples for both groups are listed in the electronic supplementary material. The fracture strength and elastic modulus of transversal samples were found to be 95 ± 7 MPa and 56 ± 7 GPa, respectively. These are significantly higher than the fracture strength (69 ± 20 MPa) and elastic modulus (35 ± 6 GPa) obtained from the longitudinal samples. Bechtle [9] explained the superior mechanical properties of transversal samples based on the fact that the inner enamel is involved in the fracture process from the beginning so that some prisms and ipm layers are broken during the entire fracture process. Comparing the strength and elastic modulus of bulk enamel with bulk HAP, the values of the former are slightly lower, and this can be attributed to the presence of proteins in enamel. However, there is a one order of magnitude reduction in the fracture strength from the crystallite level of approximately 1000 MPa to the bulk enamel of approximately 100 MPa. The orientation of the structural elements (crystallites, prisms) is believed to play a major role, which is highly complex in bulk enamel, and controlled in the small-scale samples shown in figure 4. In the latter, crystallites and prisms were uniaxially loaded along their long axis, leading to mineral fracture; however, in the bulk samples, the majority of the prisms were loaded perpendicular to their long axis, leading to prism separation at the weaker protein boundaries. The fracture strength of micro-cantilever bending specimens, when loaded perpendicular to prism axes, was reduced to approximately 350 MPa [49], supporting our argument on the role of orientation. Moreover, according to Gao’s hierarchical model, each additional level of hierarchy adds more protein to the composite structure [16], which can also account for the much lower strength of bulk enamel. Finally, in the millimetre-sized bulk samples, it is difficult to avoid microcracks induced by successive grinding and polishing procedures. Accordingly, the amount and size of the defects
are substantially higher in bulk samples, which can also lower the strength. Nevertheless, the benefit of hierarchy at this level manifested itself in the damage-tolerant stress–strain behaviour (figure 9a). Neither of the bulk sample groups showed a catastrophic fracture but failed in a gradual manner resembling those of artificial fibre-reinforced composites [9]. There was a sudden drop in the stress level at fracture initiation but further loading resulted in progressive damaging of the samples. Both enamel sample groups remained macroscopically intact after testing. The crack profiles varied with respect to the prism orientation. In the transversal sample, multiple macroscopic cracks spreading over a large area were evident (figure 9b); however, there was a primary crack that propagated straight through the outer enamel which became highly tortuous within the inner enamel of the longitudinal sample (figure 9c).

In general, apparent fracture toughness values obtained by different methods corresponding to the hierarchical level 1 and level 2 lie roughly between 0.5 and 1.5 MPa m$^{0.5}$. At hierarchical level 3, radial and decussating enamel were analysed separately and both exhibited rising crack growth resistance, whereas R-curve studies at hierarchical level 4 investigated the synergistic effect of radial and decussating enamel and the role of their spatial locations. Bechtle et al. [10,11] examined the role of prism orientation (transversal and longitudinal) and crack growth direction (from outer to inner enamel and the reverse direction) using single edge notched (SENB) bulk bovine enamel samples tested in three-point bending. All cracks grew at an oblique angle of approximately 45° from the initial notch direction independent of sample orientation with the crack plane always being perpendicular to the side surface (as illustrated with the red line on the schematic drawing in figure 10). This introduced mixed-mode stress conditions at the crack tip, mode I (tensile) and mode II (in-plane shear) stresses. The determined $K_{\text{IR}}^*$ and $K_{\text{IIR}}^*$ values increased with increasing crack extension in all sample groups (asterisk indicates that this is not a fracture resistance in the usual sense because of the lack of a known fracture criterion for enamel under mixed-mode loading). The initiation and maximum fracture toughness values obtained from the R-curves are listed in the electronic supplementary material, table S2.
values were always about one-quarter to one-third of the $K_{IR}^*$ values for all orientations tested by the same amount of crack increment and therefore only mode I fracture toughness resistance curves are shown in figure 10. Also included in the graph are the corresponding crack lengths (approx. 50 µm), the indentation toughness values obtained from enamel that correspond to the initiation toughness at crack growth $K_{IR}^* = 1$ MPa m$^{0.5}$ of the SENB specimens. $K_{IR}^*$ values rise to 4–4.5 MPa m$^{0.5}$ after 300–500 µm crack extension in transversal and longitudinal (crack growth from outer to inner enamel) samples. However, in the other longitudinal samples with crack growth from inner to outer enamel, the fracture toughness increased only to 2.5–3.0 MPa m$^{0.5}$. Similarly, R-curve measurements performed by CT specimens revealed (results listed in the electronic supplementary material, table S2) that enamel exhibits an increase in the fracture resistance in both directions, but cracks growing from inner to outer enamel were clearly less stable [7,13]. The reasons for higher fracture resistance of cracks propagating from outer to inner enamel are considered in the electronic supplementary material.

Bajaj & Arola [7] estimated the contributions of the dominant toughening mechanisms to the fracture toughness of enamel using theoretical models. The authors did not specify the contributions according to the size scales but only according to the mechanisms. Unbroken mineral ligaments have the highest contribution to toughening of enamel with bridging stress intensity between 0.2 and 0.6 MPa m$^{0.5}$, which is approximately 25% of the global toughness in their study [7,13]. The reduction in the local stress intensity factor induced by bridging of organic matrix was estimated to be 0.1 MPa m$^{0.5}$. The contribution associated with microcracking was estimated to range from 0.38 to 0.49 MPa m$^{0.5}$.

3. Conclusion

The hierarchical microstructure of enamel and the research on its fracture behaviour were reviewed by classifying the results from independent studies according to their corresponding hierarchical levels. This provided an overview of the influence of hierarchical organization on the fracture toughness, fracture strength and deformation behaviour quantitatively. The contribution of the structural features to the mechanical results was specified for each hierarchical level. Overall, these results indicate that there is a sharp decrease in fracture strength from nano-scaled HAP crystallites to bulk enamel containing all levels of hierarchy. But, there is
also a transition from brittle to quasi-ductile deformation behaviour indicating the damage
tolerance of enamel acquired by increasing hierarchy. This is also supported by the fracture
toughness investigations showing R-curve behaviour measured at higher hierarchical levels.
Toughening mechanisms including microcracking, protein and mineral bridges, crack deflection
and branching are identified at almost all hierarchical levels. It is believed that they act
simultaneously on the crack, resulting in growth resistance of enamel, but the contribution of
these mechanisms increases with increasing number of levels owing to the enlarged size of the
structural elements. Apart from the structural mechanisms gained by hierarchical organization,
increasing number of levels facilitates higher protein content as suggested by Gao’s hierarchical
model, which is however not as yet directly quantifiable. Although the quantitative contribution
of crack bridges formed by proteins is found to be insignificant [7], their benefit is postulated
to be limited slippage that could reduce stresses without crack growth and recovery (closure) of
cracks [53,69,70]. If there were no proteins, then there would be greater damage accumulation
and break-up of the microcracked structure, especially under multiple contact loading, which
is not frequently observed. With age, as the protein breaks down and is not repaired, then
enamel tends to become more brittle [71]. Overall, enamel consists of nanoscale building blocks
and weak interfaces over various length scales and has acquired, with evolution and species-
dependent nutrition, a complex architecture. This enables enamel to arrest cracks and to channel
them along configurations which eventually impede their propagation [72]. This strategy is
designated as ‘crack trapping and locking’ by Mirkhalaf et al. [72] and has more recently been
implemented in glass structures. The deformability of the glass was improved by generating weak
interfaces—through three-dimensional laser engraving—that were infiltrated with polyurethane.

The complex nature of the microstructure and resultant mechanical response of enamel,
especially crack growth behaviour with increasing hierarchical level, remains an area of fertile
research. In this study, only quasi-static crack growth has been investigated, whereas biologically
enamel experiences complex repetitive stresses as individual teeth are loaded. In addition, the
oral environment, with its range of pH, presence of proteases that may assist with break-up
of the remnant protein coupled with the current widespread usage of bleaching procedures
that fragment these proteins, may have an influence on the resultant properties, especially of
the higher hierarchical levels. The clinical implications of these aspects along with the role of
various clinical conditions such as enamel caries, hypo-mineralization, fluorosis and others awaits
further investigation.

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