1. Introduction

Enamel is the hard outer coating protecting the soft dentin interior in teeth. It is the hardest part of the human body. These critical load bearing tissues often survive in the oral environment millions of loading cycles in the form of compression, wear and torsion where the critical stress could range up to 2.5 GPa [1]. Its functional success is a desired feature for loading structures. However, a deep understanding of enamel's structure-property relationship is essential before the principles could be applied to advanced structural materials design. Besides, in clinical perspective, a better understanding of enamel's structure-property relationship provides a basis for improvements in restorative as well as preventive dentistry.

Teeth enamel's resilience is also somewhat surprising. 90% by volume of its structure consists of biological apatite crystallites that are as brittle as soda-lime glass (enamel's apatites extracted from de-proteinization have an average fracture toughness of $\approx$1.0 MPa$\cdot\sqrt{m}$ [2] whereas the fracture toughness of soda-lime glass has been measured as 0.7-1.2 MPa$\cdot\sqrt{m}$ [3]), the rest constituents are compliant organics (as soft as skin) and water [4]. After decades of extensive study of their functionality and structure-property relationship [5], it is today still a rather controversial area. Firstly, enamel is traditionally considered as having little chances to deform and its fracture absorbs relatively low energy compared to the underlying dentin [6]. But the fact is that enamel seldom fails catastrophically except in extreme trauma conditions. Recent studies have

even shown that enamel is able to imply toughening mechanisms after crack-initiation [7–9]. Secondly, it is speculated that proteins contribute to energy absorption leading to enamel's toughness [10]. Modeling [11] studies and experimental results from protein molecules in bone [12, 13] support this argument but there is little such microstructure-specific experimental evidence in enamel. Some even claim that the existence of very low amount of enamel proteins 'likely do not play any major structuring function' [14]. Thirdly, calculations show that the levels of hierarchical structure inside the materials does not necessarily correlate to the excellence of mechanical properties [15, 16], whereas some show that tailoring the elementary building blocks at nanoscale is indeed essential for the strength to achieve theoretical values [11]. Lastly, enamel's properties often show a wide variation of values [1].

Therefore, this study is dedicated to improve the fundamental understanding of enamel's structure-property relationship. What are enamel's mechanical properties in relation to its length scales? Does the small amount of proteins play a crucial role in dissipating energy? What is the correlation between enamel's multiple hierarchical levels of structure with its mechanical properties and its role in maintaining the structural integrity? The main characterization tools in this study are nanoindentation and atomic force microscopy.

Although the overall teeth survival could also be due to the synergy of the co-existence between enamel and dentin, the graded junctions between these tissues and/or the shape of the teeth, this study focus to explore the multi-scale properties of enamel itself to understand its secrets of functionality. If enamel itself has not been a high-performing structure, it could not have survived as the outermost coating layer of an important loading structure, teeth.

2. Theoretical Basis

In this chapter, the theoretical basis of the materials, the equipments and the relevant analyses are explained. In Chapter 2.1, the hierarchical structure of enamel, its formation process and its compositions are summarized. The analysis of nanoindetation data is detailed in Chapter 2.2. In Chapter 2.3, the basic principles of fracture mechanics for brittle solids (such as linear elastic fracture mechanics and the non-linear processes around the crack tip) are explained.

2.1. Enamel

A detailed literature review is done to understand the structure, the development process and the compositions of human enamel.

2.1.1. The hierarchical structure of human enamel

A longitudinal section of a human tooth is shown in Fig. 2.1 (a). Enamel rods are closely packed and extend from DEJ to near enamel surface (Fig. 2.1 (b)), estimated to be 6-12 μm below the tooth surface [17]. The rods are embedded in a network of interrod structures (Fig. 2.1 (c)). The diameter of the rods increases from ≈3 μm in the

inner enamel to ≈ 6 µm in the outer enamel [17]. Enamel rods are partially demarcated by organics.

Each rod as well as the interrod regions consist of apatite crystallite fibers of 24-35 nm in thickness and 55-90 nm in width [18, 19]. They have irregular outline (Fig. 2.1 (d) and (e)) as they are pressed against each other during crystallites growth [14]. The crystallites are 40-50 µm long, and some researchers believe that they span over the entire thickness of the enamel layer [14]. Each crystallite is enveloped by an ≈1 nm thick organic layer [18].

The crystallographic axis of the crystallite fibers lies along their longitudinal axis [20], Whereas the orientation of crystallite fibers relative to the rod axis depends on its location around and inside a rod (Fig. 2.2). Within a rod, crystallites are mostly oriented along the rod axis, but the further away their locations from the rod edge with U-shaped organic sheath, the more the deviation of their orientation in comparison to rod axis. This variation of orientation is up to 50 degrees and has been observed in a previous study [21]. The crystallite orientations inside the interrod region are significantly different from the adjacent crystallites inside rods and could deviate to up to 90° [9].

The alternating bright and dark bands in enamel (marked by yellow dotted lines in Fig. 2.1) are called the bands of Hunter and Schreger [22–24]. This optical phenomenon is caused by changes in orientations between adjacent groups of enamel rods (Fig. 2.3). They mainly occur in the inner two thirds of the enamel and are also called rod decussations, where enamel rods bend to the left and right and have different local orientations while extending from DEJ towards the direction of enamel surface.

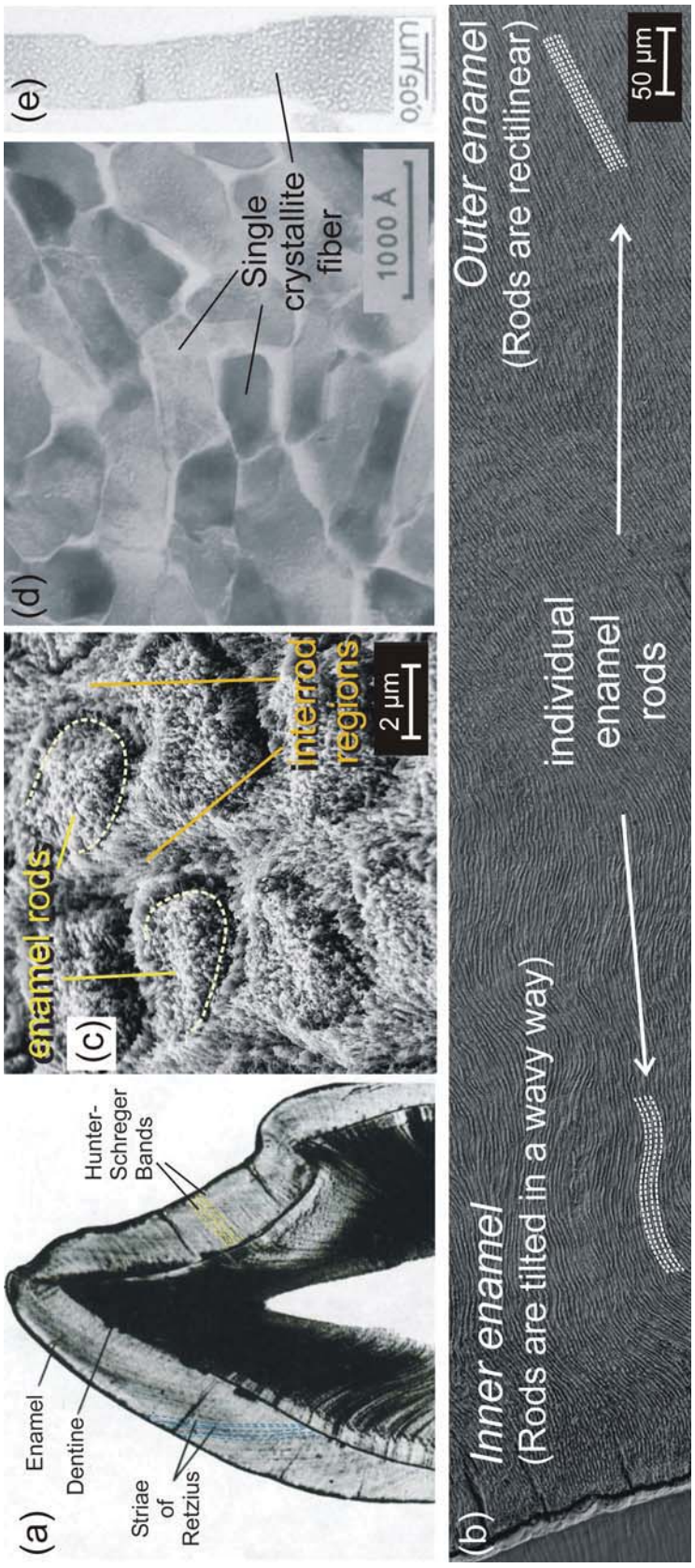

Figure 2.1.: The structure of enamel. (a) A longitudinal section of a tooth and its Hunter-Schreger bands (for magnification, see Fig 2.3) and the lines of Retzius [17]. (b) Enamel rods are closely packed and span from inner enamel to outer enamel region. The rods in the inner region are more intertwoven. (c) The enamel rods and the interrod region. (d) The cross sections of enamel crystallite fibers [18]. (e) Enamel crystallite fibers are at least 100 µm long and have irregular outline [25].

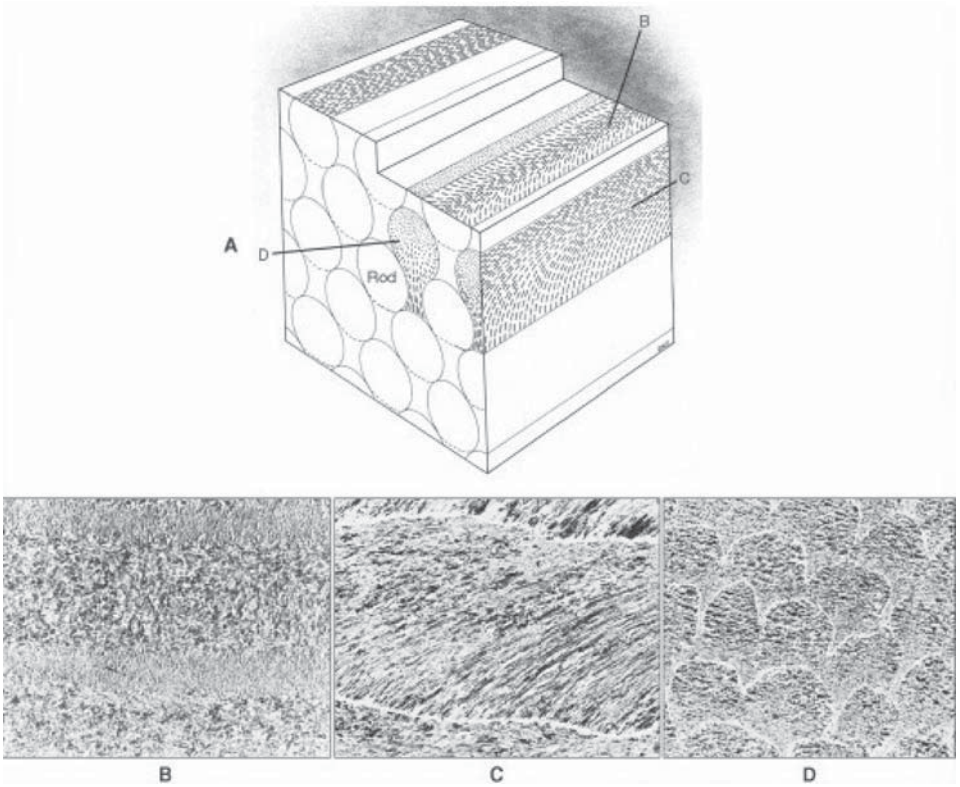

Figure 2.2.: Crystallite fiber orientations inside enamel rods. Figure A shows a 3 dimensional illustration. Figure B-D shows TEM images of the 3 faces in A [14, 21].

The series of dark lines (some are marked by blue dotted lines) in Fig. 2.1 (a) are called the striae of Retzius [26]. These are light microscope manifestation due to rhythmic swelling and shrinking of the rod diameter [17]. These intervals are about 4 μm in width. They are largely attributed to a weekly rhythm in enamel formation [26]; some suggest that each line of Retzius separates different cohorts of cells that are grown side by side [14]. The lines of Retzius end at the enamel surface as shallow trenches known as Perikymata, visible on newly erupted teeth [27].

Since enamel is a hierarchical-structured material, its structure can be described in terms of a 0^{th} to 3^{rd} hierarchical level, where the smaller structural elements compose bigger structural level and so forth [15, 16]. The ≈50 nm diameter apatite crystallite fibers are the mineralized structural elements of the 0^{th} hierarchical level. Groups of

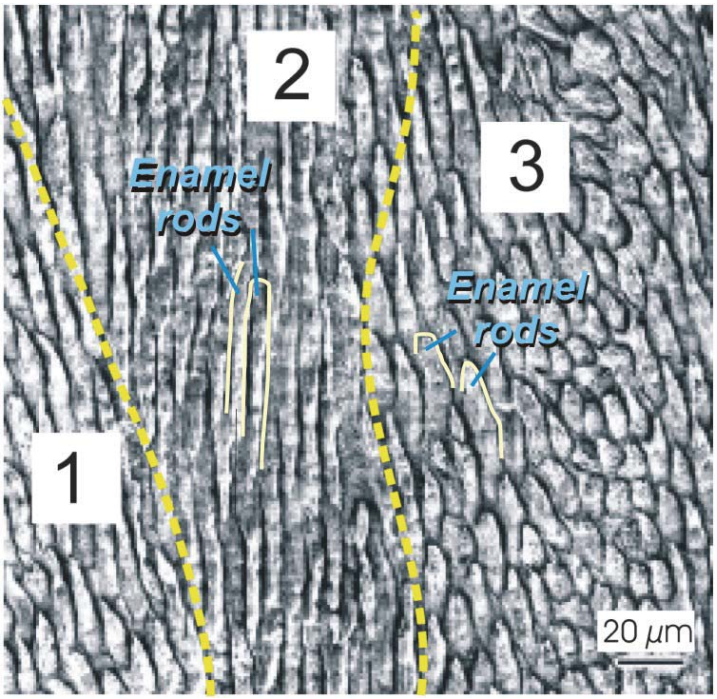

Figure 2.3.: An SEM image illustrates three adjacent Hunter-Schreger bands [14]. Each Hunter-Schreger band represents a group of enamel rods of similar local orientation but different from those in the adjacent Hunter-Schreger bands.

apatite crystallite fibers are bundled together within one enamel rod or in the interrod region as the 1st hierarchical level. The enamel rods are grouped to form the 2nd level in Hunter-Schreger bands in the inner enamel rod region. The rods within each band have the same local orientation but different from the orientation of enamel rods inside the adjacent Hunter-Schreger bands. Multiple Hunter-Schreger bands form the 3rd hierarchical level as bulk enamel.

2.1.2. Enamel formation

This section is summarized from Chapter 7 - Enamel: Composition, Formation and Structure in the book Ten Cate's Oral Histology by Antonio Nanci [14]. In this chapter, italic forms are used for all scientific terms relevant to enamel formation because these are generally non-familiar terms for materials scientists.

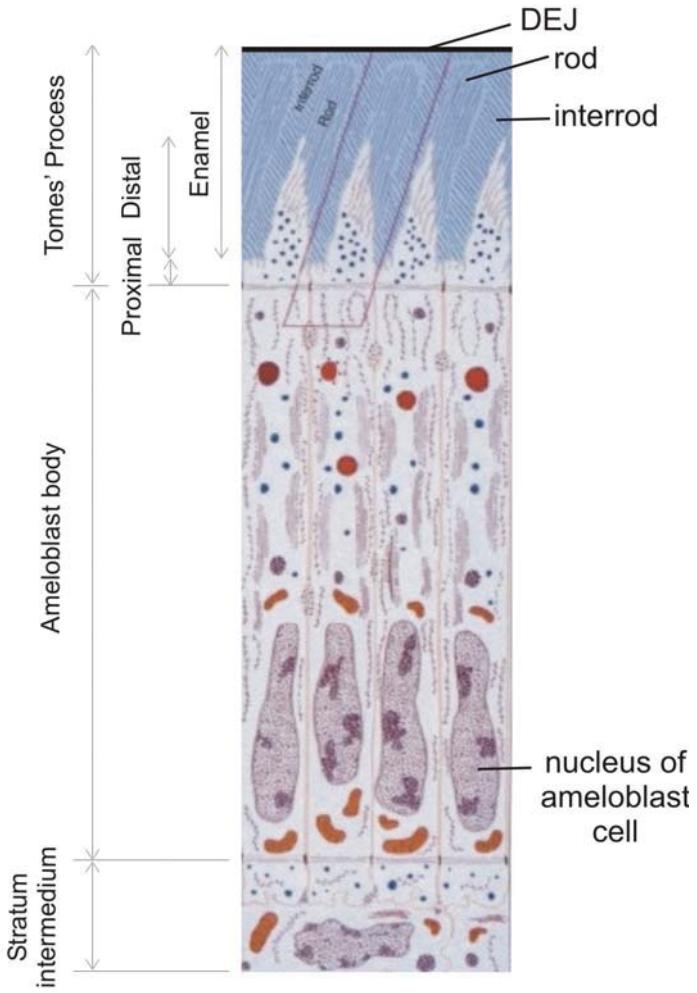

Figure 2.4.: A schematic representation of four ameloblast cells in a section along their long axis, showing (from bottom to top) stratum intermedium (believed to be closely related to the development of ameloblasts), ameloblasts' bodies, ameloblasts' proximal portion of Tomes' Process (ppTP), ameloblasts' distal portion of Tomes' Process (dpTP) and the growing enamel layer. [14].

The process of enamel formation is called *amelogenesis*. *Ameloblasts* are cells from which tooth enamel develops. *Ameloblasts* (Fig. 2.4) are compartmentalized into body and *Tomes' process*. Mineral deposition is accomplished at Tomes' Process and begins at the dentinoenamel junction (DEJ) and ends at the outer enamel, constantly pushing the *ameloblasts* away from the DEJ. The non-secreting end of ameloblast cells are attached to the *stratum intermedium*. Crystallite formation is accompanied by enamel protein secretion (discharge) out of *ameloblasts* and are segregated at two sites: (i) around the

periphery of the *ameloblasts* on the *ppTP* (proximal portion of Tomes' Process) and (ii) along the face of the *dpTP* (distal portion of Tomes' Process) (Fig. 2.4). The crystallites growth at *ppTP* forms *interrod* regions whereas those at *dpTP* forms *rods*. The formation of initial and final enamel layers are formed by the *ppTP* surfaces only (without *dpTP*) and therefore contain no enamel rods. After crystallite formation, enamel then hardens by the growth in width and thickness of pre-existing crystallites while the matrix proteins and enamel fluid diminish (maturation stage). During the maturation stage, no new crystallites are formed.

The proteins identified in enamel include *ameloblastins*, *enamelins*, *amelogenins* (90% of the proteins), *tuftelins*, *enamelysin*, *KLK4*, *amelotin* and *Apin*.

Based on the biochemical characteristics, enamel proteins *ameloblastin* and *enamelin* are believed to guide the formation of enamel crystals. They undergo rapid extracellular processing and have short half lives. Therefore, their small amount does not necessarily mean that they are produced in small amounts but might actually mean that they do not accumulate over long periods. Those found in the deeper enamel are mostly degraded fragments whereas those near enamel's surface are more intact.

Amelogenin makes up 90% of enamel proteins. They are believed to form 'nanospheres' surrounding crystallites along their axis. Based on the biochemical characteristics and their distribution, they are believed to regulate growth in thickness and width of crystallites. They are hydrophobic and are rich in *proline*, *histidine* and *glutamine*. They undergo extracellular processing by enzymes into smaller fragments, *tyrosine*- and *leucine*-rich *amelogenin* polypeptide comprises the bulk of the final organic matrix.

Enamelysin and *KLK4* are involved in the extracellular processing and degradation of the enamel matrix. *Enamelysin* is involved in the short-termed processing of the

newly secreted matrix, whereas *KLK4* acts as a digestive enzyme especially during the maturation stage. The role of *tuftelins* in the enamel matrix is still unclear.

The enamel proteins *amelotin* and *apin* are associated with the modulating surfaces between ruffled-ended and smooth surfaces of *ameloblasts* during maturation stage. The cyclic modulations between creation, decline and recreation of a ruffle-ended borders or smooth surfaces of *ameloblasts* is hypothesized to maintain good pH conditions for mineralization and matrix degrading processes.

Amelogenesis can take as long as 5 years to complete, and about two thirds of the formation time is dedicated to the maturation stage. After the maturation stage, the tooth awaits eruption through gum bed into the oral cavity.

2.1.3. Compositions of human enamel

Human enamel contains ≈96% by weight of mineral [28], individual values range from 93.6-98.5% [29–39]. 0.05-8% of its composition is organic matters [35, 39–47], averaging at approximately 0.5% [28]. The rest of the composition is water. By taking the density of minerals, organic matters and water as 3.0, 1.4 and 1.0 g/cm^3, the % by volume for these constituents are calculated as 90%, 2% and 8% (Tab. 2.1) [4, 28].

Table 2.1.: Approximate content of main constituents of sound human enamel [4, 28].

Constituent	**% by weight**	**Density (g/cm^3)**	**% by volume**
Mineral	96	3.0	90
Organic matter	1	1.4	2
Water	3	1.0	8