REVIEW ARTICLE AGE RELATED CHANGES IN PHYSIOLOGY OF NORMAL HUMAN TOOTH ENAMEL: A REVIEW

Afsheen Mansoor, Faisal Moeen*, Aleena Hussain**, Shahab Ud Din, Muhammad Talal Khan***, Farrukh Said*

Department of Dental Material, School of Dentistry, Shaheed Zulfiqar Ali Bhutto University, *Islamic International Dental College, Riphah International University, **Student, Islamabad Medical and Dental College, Islamabad, ***Bakhtawar Amin Medical and Dental College, Multan, Pakistan

There are usually 32 teeth in adult humans which are separated equally into two arches namely the upper arch (maxilla) and the lower arch (mandible). Dental enamel is the outermost protective layer that aids the tooth in bearing the masticatory forces and helps it endure the harsh oral environment. Age-related changes occur in teeth approximately between ten weeks in utero to old age. Lacassagne in 1889 was the first to characterise changes in fully formed teeth with ageing; however, the first scientific assessment was provided by Gustafson. In 1967, Furuhata and Yamamoto added various phenomenon that may be expedient in defining an age. After twenty-five years of age, they linked an increase in the specific gravity of teeth with age. They further pointed out the process of attrition, which progresses with age, and the components of the tooth namely enamel, dentine, and cementum harden with increasing age. These investigators also defined the decreasing size of the root canals with age. Bodecher and Lefkowitz employed permeability and x-ray studies to indicate maturational effects in the human teeth. Kastelic determined through the incineration studies, an increase, upon a percentage basis, in the organic content of the whole human tooth with age. Ultimately age-related changes in teeth are based on biological markers of age. Teeth reflect the biological or physiological age of the individual and variations caused by genetic factors and chewing habits can influence tooth anatomy.

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HUMAN TOOTH

There are usually 32 teeth in the adult human which are separated equally into two arches namely the upper arch (maxilla) and the lower arch (mandible). Depending on their position in the arch, teeth consist of incisors and canines which aid in cutting and shearing of the food, while the posteriors consist of premolars and molars which aid in the digestion of food.

Each human tooth is anatomically separated into a crown and one or more roots. The anatomical crown is composed of an inner soft tissue referred to as pulp chamber that is enveloped by a relatively harder tissue called dentine that is further surrounded by one of the hardest tissues in the human body known as enamel.¹ The junction between the pulp chamber and the dentine is called dentino-pulpal junction (DPJ), whereas the junction between the dentine and enamel is known as a dentino-enamel junction (DEJ) (Figure -1).

Human Tooth Enamel

Dental enamel is the outer most protective layer that aids the tooth in bearing the masticatory forces and helps it endure the harsh oral environment.² Mature dental enamel is a complex structure made up of mainly inorganic (96% w/w) and a small fraction of organic material and water (4% w/w) and contains no cells and collagen.³ The enamel structure is divided into seven histological levels that are highly organized and range from nano-scale to micro scale. These include hydroxyl-apatite nano-crystals (~15–30 nm), nano-fibrils (~30–40 nm), fibrils (~80–130 nm), fibres (~80 nm), prisms (~1 μ m), key-hole shaped structures (~6–8 μ m) and prism bands (~100 μ m).¹

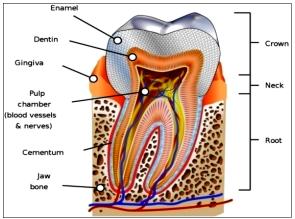


Figure-1: Structure of human tooth.

Hierarchical Structure of Human Enamel Hydroxyapatite crystals, the basic structural blocks of human tooth enamel, elongate along a preferential axis and aggregate to form nanofibrils. These fibrils in the human tooth enamel are unique structures that always run lengthways concerning each other and aggregate into thicker fibrils. These fibrils are well packed and form enamel fibres that also aggregate into bigger structures called enamel rods or prisms.¹ The highest level of organisation is prism bands that run parallel to each other and are arranged in different patterns along the entire thickness of the enamel.¹

In the longitudinal section, bands of prism in outer third portion of enamel are named as radial enamel. These are radially oriented which intercept with the outer surface of enamel in the perpendicular direction. Hence these bands are truly responsible for the striae of Retzius lines phenomenon.¹ However, in the inner regions the bands of prism are oriented in more perpendicular fashion which are basically responsible for the bands of Hunter-Shreger phenomenon.^{1,4} When the bands of prism are cut cross-sectionally, they are evident as well packed key-hole shaped structures that are $\sim 6-8 \mu m$ in size. However, they encompass the numerous packed prisms and the inter-prisms which are separated by the organic rich sheath having no crystallites.¹ Prisms of enamel protrude in the perpendicular direction from dentinoenamel junction, i.e., DEJ to enamel surface thus running somewhat parallel to each other.⁵ Hence, long c-axis of the crystallites that form prism is parallel to long axis of prisms, where as crystallites around each prism tilt at about 40° – 65° relative to direction of prism thus forming interprisms.⁴

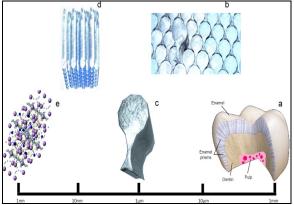


Figure-2: Hierarchical structure of human enamel

Organic Component of Human Enamel

Enamel is avascular and has no nerve supply within it. Moreover, it cannot be renewed. The organic component of mature enamel consists of 60% proteins and 40% lipids.⁶ The enamel proteins include noncollagenous phosphorylated proteins: amelogenin, enamelin, hamelin, ameloblastin and proteinases. Immature enamel contains a higher amount of proteins which have an important role in the development of enamel. As the enamel matures, the total amount of proteins decreases and the organic portions of the mature enamel become concentrated mainly at the prism sheath and to a lesser amount between the crystallites.⁷ Due to the semi-permeable nature of the organic part of enamel, it has been assumed that it acts as a channel that allows the small molecules such as bleaching agents to penetrate the enamel.⁸

Mineral Component of Human Enamel

Tooth enamel is the most mineralised tissue in the human body. Enamel consists of about 95% mineral by weight, but 87% mineral by volume. Enamel mineral is composed of well packed calcium-phosphate apatite nanocrystals. The mineral phase of enamel is compatible with that of pure stoichiometric hydroxyapatite with a general formula Ca^{10} (PO4)⁶ (OH)^{2.9} Furthermore, it is not a static tissue as it can undergo deviations in mineralisation.

crystals Basically Hydroxyapatite are hexagonal which belong to space group P63m. It has two cell lattice parameters, i.e., a=b=0.942 A° and c =0.688 A° . In this case 10 calcium ions, i.e., Ca^{++} are located in two different non-equivalent crystallographic sites in structure of apatite.^{9,10} (Figure-2). Four of these calcium ions are found at position named as Ca (1) which are organized in columnar manner surrounded by 9 oxygen (O) atoms that belong to 6 phosphate tetrahedral PO_4^{3-} . Other 6 Ca ions are found at position named as Ca (2) which are organized in 2 set of triangles encircled by 6 Oxygen atoms belonging to 4 PO₄³⁻ and 1 Oxygen atom belonging to hydroxyl ion. Thus, these two triangles are arranged in such a way that they form a channel named as anion channel along caxis that are occupied by 2 OH per crystal unit.

Similar to the pure hydroxyapatite crystals the dental enamel crystals are also hexagonal structures but they are having large cell lattice parameters, i.e., $a=b=9.433 \text{ A}^{\circ}$ and $6.896 \text{ A}^{\circ,9}$ Thus, enamel crystals are best named as carbonated crystals hydroxyapatite, i.e., CHA.¹¹

The inorganic elements of dental hard tissues contain not only calcium (Ca), phosphorus (P), and oxygen (O), as shown in the formula of calcium hydroxyapatite, but also carbon (C), magnesium (Mg), sodium (Na), fluoride (F), potassium (K), zinc (Zn), lead (Pb), nitrogen (N), iron (Fe) as well as other numerous trace elements.^{6,12} As such, these minerals are joined into the enamel by ion exchange, by absorption at the crystal surface and by absorption in the hydration layer between the crystals.¹³ A large amount of mineral in the enamel makes up for its strength and brittleness.¹⁴

Ionic Substitutions in Enamel Apatite Crystal Enamel Apatite

Basically, like other biological apatites the enamel apatite is capable of accommodating specific ionic or chemical substitution because it is nonstoichiometric and poor crystalline material.¹⁰ Ionic exchange can

occur through the different sites available in the crystal structure of apatite which allows the incorporation of several different ions.¹⁶ Thus, certain criteria must be fulfilled by the ionic substitutions: Substituting ions must maintain a certain level of geometric parameters to be fitted in crystal lattice and they should also maintain the overall charge balance of the lattice of crystal.¹⁵ The most common ionic substitutions in the apatite crystal are iron ions (Fe⁺⁺), Cl⁻, CO₃⁻⁻, F⁻, Na⁺ and Mg^{++,1,15} Small quantities of ionic substitutions in structure of apatite alters the characteristics of minerals thus producing main effects on physical and chemical properties of apatite, i.e., hardness, thermal stability and solubility.¹¹

Carbonate and Fluoride

High carbonate (CO_3^-) content of approximately 2–5 wt% is found in dental enamel.¹⁶ Basically, Carbonate is a large anion which is about 90–95% of CO_3^- that replaces the phosphate tetrahedral PO₄⁻⁻, i.e., type B substitution. On the other hand, 5–10% remaining CO_3^- is replaced by OH⁻, i.e., type A substitution.¹⁶ Due to this reason, it is found that increased content of carbonate in the dental enamel leads to the increased caries susceptibility and solubility¹⁷, decreased crystallinity^{16,18} and microhardness.¹⁹ Moreover, fluoride is replaced by OH which is considered to have the outstanding fit in the site of anion channel.¹⁹ Thus, Fluoride incorporation in the enamel enhances its resistance to demineralization.²⁰

Magnesium

Magnesium (Mg) is the most abundant ion found in the mineralized tissues of human body which comprises of approximately 0.44 wt% of enamel, 0.72 wt% of bone and 1.23 wt% of dentine.¹⁰ In the mature human dental enamel, the concentration of magnesium vary from about 0.4% near dentino-enamel junction, i.e., DEJ and then decreases to 0.1% near the surface of enamel.²¹ With the advancing age, magnesium levels in body tends to drop from body stores. Thus, Mg deficiency produces a major influence on the mineralized tissues by causing defects in their physical properties, growth and metabolism.¹⁵ However, the content of Mg ions in human dental enamel's fluid that surround the new forming crystals play an important role during the development of human tooth enamel and degree of uptake of Mg ions regulates the dental enamel mineralization.^{22,23}

Dental Enamel Hardness

The hardness of a material is a property that is used to describe its resistance to plastic deformation, scratching or indentation. Dental enamel is considered as the hardest biological structure in the human body, and this property allows it to endure the harsh oral environment.² The hardness of the tooth enamel has been widely

investigated in the literature and has been utilised as an indicator to assess other tooth properties, such as enamel demineralisation, caries susceptibility, wear resistance and crack propagation.²⁴

It varies between different individuals and between different teeth within the same individual. It has been postulated that age, prism orientation, crystal size, crystallinity, organic matrix content, water content and inorganic carbonate content might affect the enamel hardness as well.²⁴ Average hardness for enamel ranges from 250–360 VHN.²² Wilson and love testified these values of micro-hardness for enamel and reported ranges from $263\pm26-327\pm40$.

Natural Tooth Shade

Human teeth play a very major role in the overall facial appearance that totally affects the individual's self-confidence altogether with the social life.²⁵ The appearance of the human teeth totally depends on their colour, shape and the alignment in arch. Thus, the understanding of human tooth color is important and essential for many different aspects in the clinical dental practice, particularly in the cosmetic dentistry as well as the restorative dentistry.

The overall tooth shade represents a combination of the natural shade of dental tissues (enamel, dentine, pulp) and the extrinsic stains that are adsorbed on the surface of the dental enamel.²⁵ It has been suggested that the crystal size of carboxy hydroxyl apatite (CHA) of enamel has a major effect on the tooth shade parameters by influencing the scattering of light that occurs on the tooth surface smaller crystals scatter more amount of light making the teeth appear whiter.²⁶

The shade of human teeth is made of a combination of colors that vary from light yellow to greyish white which graduate from the incisal edge to the gingival margin of the tooth. The gingival margin inclines to be more yellowish and darker, while the incisal edge tends to be more greyish and lighter.²⁵ Teeth are lighter in the younger people and primary dentition, but with the increasing age, teeth become darker in the shade.²⁵

Many properties of dental enamel have been reported to influence tooth shade, such as enamel thickness, chemical composition and crystallographic properties.

Age-related changes in Enamel

Age-related changes occur in teeth between approximately ten weeks in utero to old age.^{27,13} Lacassagne in 1889 was the first to characterise changes in fully formed teeth with ageing; however, the first scientific assessment was reported by a study.¹³ He characterized tooth aging based on a scale of the severity of attrition, gingival recession, transparency of root, root resorption, apposition of secondary cementum at the root apex, and increasing secondary dentinal thickness variations in these parameters with aging have provided a high degree of accuracy for estimating age and have been studied many times.^{28,29}

In 1967, Furuhata and Yamamoto added various phenomenon that may be expedient in defining an age. After twenty-five years of age, they linked an increase in the specific gravity of teeth with age. They further pointed out the process of attrition, which progresses with age, and the components of the tooth namely enamel, dentine, and cementum harden with increasing age. These investigators also defined the decreasing size of the root canals with age.³⁰

Bodecher and Lefkowitz employed permeability and x-ray studies to indicate maturational effects in the human teeth.³¹ Kastelic determined through the incineration studies, an increase, upon a percentage basis, in the organic content of the whole human tooth with age.¹³ Ultimately age-related changes in teeth are based on biological markers of age. Teeth reflect the biological or physiological age of the individual and variations caused by genetic factors and chewing habits can influence tooth anatomy.³²

TOPOGRAPHICAL CHANGES IN ENAMEL

Structural Change in Enamel

In the enamel, there are parts with an ailing calcified interprismatic substance such as enamel lamellae, tufts, and spindles. They consist of mainly organic compounds in the form of thin fibrous structures.³²

The enamel lamellae resemble thin leaf-like constructions that spread from the enamel surface to the dentino-enamel junction. They cover in a longitudinal direction and are more easily detected in the transverse (longitudinal) plane of the tooth where they look like fine cracks. They are not cracks but enamel tunnels which provide easier penetration of organic substances into the enamel.³³

The number of enamel tunnels decreases with increase in age; their distribution on the enamel surface becomes less, and their diameter increases up to 3 μ m. The enamel tunnels define more precisely the qualitative and quantitative microelement structure of their walls and inner content of both organic and inorganic nature.³³

Permeability Change in Enamel

In enamel maturation, critical importance is given to its permeability and solubility. There is no doubt that these parameters are bound to change during life.^{25,33} The extent of enamel permeability is different at different ages. It is a key in the non-erupted teeth, and then it decreases in the permanent teeth immediately after their eruption. Primary teeth are less porous and permanent teeth of adults have the lowest permeability.^{33,34} Therefore, at this point, the enamel is more porous^{13,32} and is more susceptible to demineralisation than mature

enamel probably because of post-eruptive maturation.^{32,33}

It is prudent to note that the enamel permeability decreases with age due to the deposition of mineral substances in the enamel coming from the saliva. The degree of enamel maturity can be calculated by taking its permeability in account.³⁵ A change in the configuration of both the mineral and organic phases of the enamel occurs with a marginal increase in age.³¹

The fluctuations in enamel permeability also hinge on the type of teeth. Incisors have the lowest permeability, and it steadily increases towards molars. As for the tooth surfaces, the labial surface is more porous than the vestibular one.³¹

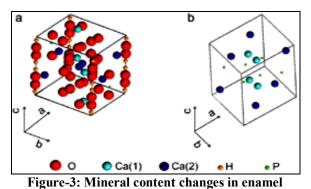
Mineral Content Changes in Enamel

Enamel does not undertake any further mineral deposition after it has been laid down by the ameloblasts. Its surface can be entirely changed by abrasion, attrition, erosion or dental caries, and at a crystal level by ion exchange, demineralisation and remineralisation. When a tooth breaks out in the oral cavity, the enamel has not yet experienced post-eruptive maturation and grants unique characteristics that make it more susceptible to demineralisation.³⁶ Therefore, at this point, the enamel is more porous with larger carbonated apatite concentration and a more significant percentage of impurities (sodium, magnesium, etc.) in its overall composition.^{36,37}

As a consequence of this, enamel crystals become more soluble in the oral environment. Hence, at eruption, the mineral structures of the enamel are vulnerable to different variations in the oral environment.³⁸ The saliva presents calcium, phosphorus and fluorine ions which are the main mineral components of the crystalline structure of the tooth that naturally protect the enamel, favouring the maturation and making it less soluble. Previous studies have shown that altered characteristics of the enamel that has not yet experienced post-eruptive maturation from that, which has undertaken this type of change in the oral cavity.^{36,37}

Though, the nature of this maturation is not well assumed. Moreover, studies have demonstrated that immature enamel is more susceptible to demineralisation than mature enamel probably because of post-eruptive maturation.³⁸

A change in the configuration of both the mineral and organic phases of the enamel occurs with a marginal increase in age. It has been indicated that a small rise in calcification occurs during aging; this increase would explain the loss of permeability and possibly making the enamel more translucent and brittle. However, small increases in the degree of calcification would not be easy to detect in such a highly calcified tissue.



Hardness changes in Enamel

A study revealed that exterior enamel hardens with age. This fact can be understood as a direct consequence of enamel mineralisation with age as this progress occurs at the outermost surface.³⁹

It is predictable that the surge in elastic modulus and hardness with age is at least partly linked with a reduction in the amount of interprismatic organic matrix. Earlier studies have highlighted the prominence of the proteinaceous matrix on energy absorption, crack extension and the fracture toughness of this tissue.⁴⁰ In fact, White *et al* assumed that the more important fracture toughness of enamel in comparison to hydroxyl apatite is connected with the unique mechanisms of toughening enabled by the organic matrix. If the rise in hardness and elastic modulus effects from a reduction in the volume concentration of the ecological model, then there may also be a decrease in the fracture toughness of enamel with age.⁴⁰

Nevertheless, the outer enamel surface exhibits increased hardness with age. By the age of 55 years and above, both the hardness and the elastic modulus of an old enamel can increase by over 12-16%.⁴¹ Kodaka *et al* and various other researchers found a moderate correlation and association between Vickers microhardness, Phosphorus and Calcium concentration in the enamel.^{21,42} The hardness of the tooth enamel is a critical physiological property of enamel, while the calcium content is an essential parameter in the mineralisation, demineralisation and re-mineralisation of the enamel.^{21,39}

Color Change in Enamel

The colour of the teeth also provides a near real chronological age. This is because of the resistance of dental hard tissues to degradation and putrefaction. The total colour effect of natural teeth is created by the combination of light which is reflected and scattered by tooth enamel and the underlying dentine layer. Light refraction and scattering properties of the enamel can affect the perception of tooth colour.⁴³ It is basically the chromatic characteristics of enamel, dentine and pulp that determines the tooth colour.⁴⁴ Teeth can be become less white when the enamel becomes thinner and more

transparent, and the colour of the underlying dentine becomes more dominant. Characteristically colour changes in dentine, vary from white to yellow. Dentine layer thickens with age. The enamel that covers the teeth gets thinner with time and age, which enables the dentine to show through.⁴⁵ This provides a sound base for estimation of the age of an individual.

Paravina stated that human teeth are predominantly light, whitish-yellowish and slightly reddish. Increase in the darkness of the teeth is associated with aging. In the middle of a massive array of genetically determined tooth colours, all teeth darken over time along with deterioration.⁴⁶ In general, the anterior maxillary teeth are slightly more yellow than the mandibular anterior teeth, and the maxillary central incisors are advanced in value than the lateral incisors and canines.⁴⁷

No significant differences have been reported in tooth colour between males and females according to some studies involving natural teeth.³³ In general, natural tooth colour has a significant tendency to increase with the age of the subject, generally becoming darker and more yellow. With the advancing age, it has been reported that the colour can increase in redness at the incisal site because of the long term occlusal wear loss in the incisor region.⁴⁸

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Address for Correspondence:

Dr. Afsheen Mansoor, Assistant Professor, Department of Science of Dental Materials, School of Dentistry, Shaheed Zulfiqar Ali Bhutto University, Islamabad, Pakistan. **Cell:** +92-321-5879166 **Email:** drafsheengamar@gmail.com

inan: araisieenquinar@ginan.eei

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