REVIEW ARTICLE

BIOCHEMICAL BASIS OF DENTAL AGING

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To my grandmother,

For the understanding, affection and encouragement

At all times.

Aknowledgements

To Prof. Dr. João Miguel Silva e Costa Rodrigues,

for consented to guide me on this review and for all the help that he gave me during the course of the same.

To my father, António Igreja,

for giving me the chance to graduate as medical dentist, overcoming all difficulties and obstacles of being a single parent.

To my sister Paula,

for supporting me, all the time.

To all my fellow year,

that contributed to my growth as medical dentist and for all the moments of joy that provided me during these years.

“Age is an issue of mind over matter. If you don’t mind, it doesn’t matter.”

Mark Twain

Content

Abstract

Old people represent a high percentage of worldwide population. As the population ages, the demand for health care professionals who know the particularities of aging and care for the elderly, also grows. With the improvements in preventive dentistry and the decline of caries in young individuals, the number of fully dentate patients is increasing.

The teeth show signs of changes with age, mainly in their form and color. The color change is a direct consequence of aging.

Changes in biochemical and physiological processes with aging occur in all body tissues. Although most living tissues have the capacity to repair or renew themselves, the dentition is a notable exception. Given enough use for long enough, the crowns of the teeth must wear away as inevitably as the moving parts of a machine.

The dental tissues undergo age-related changes that could be ascribed to physiological, defensive, or pathological irritant-induced changes. These changes are regulated by cell activities and by a variety of macromolecules that play important roles in growth regulation, tissue differentiation and organization, formation of calcified tissue, defense mechanisms and reactions to inflammatory stimuli.

Clinically, it is important to recognize these changes and to develop planning strategies which take account of them. Emphasis must be placed on preventive regimes and treatment delivery which is sympathetic to the demands of our existing elderly and aging population.

The aim of this revision is to better understand the biochemical changes that underlie the modification of the dental structure in the aging process.

Keywords: aging, oral biochemistry, teeth structure, teeth composition, enamel proteins, dentin proteins

Resumo

Os idosos representam uma elevada percentagem da população mundial. Enquanto a população envelhece, a necessidade de profissionais de saúde que conheçam as particularidades do envelhecimento e cuidados aos idosos, também cresce. Com os avanços na área da dentisteria preventiva e a redução do aparecimento de cáries na população jovem, o número de pacientes com dentição completa está a aumentar.

Os dentes mostram sinais de mudanças com a idade, principalmente na forma e cor. A mudança de cor é uma consequência direta do envelhecimento.

Mudanças nos processos bioquímicos e fisiológicos ocorrem em todos os tecidos do corpo humano com o envelhecimento. Embora a maioria dos tecidos vivos tenham a capacidade de se reparar ou renovar, a dentição é uma notável exceção. Também as coroas, devido ao seu uso durante o tempo acabam por sofrer desgaste como qualquer componente móvel de uma máquina.

Os tecidos dentários são submetidos a mudanças relacionadas com a idade que podem ser de atribuição fisiológica, defensiva, ou alterações patológicas por estímulos nocivos induzidos. Estas mudanças são reguladas pela atividade de células específicas e por uma variedade de macromoléculas, que possuem papéis importantes na regulação do crescimento, diferenciação e organização dos tecidos, formação de tecido calcificado, mecanismos de defesa e reações a estímulos inflamatórios.

Clinicamente, é importante reconhecer estas alterações e desenvolver planeamentos estratégicos que as tenham em conta. O ênfase, deve ser dado aos regimes de prevenção e tratamento que seja adequado à evolução das necessidades da população idosa e em contínuo envelhecimento.

O objetivo desta revisão é entender melhor as alterações bioquímicas que sustentam a modificação da estrutura dentária e consequentemente o seu progressivo envelhecimento.

Palavras-Chave: envelhecimento, bioquímica oral, estrutura dentária, composição dentária, proteínas esmalte, proteínas dentina.

Introduction

The lifetime preservation and maintenance of functional teeth is a prominent aspiration of the dental professionals.

In this context, it is necessary to understand the precise nature of changes in functional tooth histology and repair activity with age, as these influence the diagnosis, planning, and provision of oral health care to fulfill these demands.

Age-related changes occur in teeth between approximately 10 weeks in *utero* to old age. [1, 2]

Many of the biochemical and tissue morphology changes are observed in normal healthy individuals. However, as people age, there is a cumulative history of past diseases and usually older people receive some kind of medication on an intermittent or continuing basis. These factors may difficult the dissociation between changes that occur due to aging *per se* and the changes due to disease or pharmacological intervention.

The study of aging includes not only the observation of changes in healthy subjects as they grow older, but also investigations on the mechanisms or causes of aging.[3]

Most recently, interest has been focused on biochemical studies of dental tissues as one of the most rigorous and reliable procedures to estimate age. Thus, a detailed knowledge about the biochemical composition of dental tissues is important since these elements contribute directly to the process of aging.[4]

Taken together, nowadays it is very important for a dentist to understand the processes associated with the ageing of oral cavity tissues, and, particularly, of the teeth. This will kept the dentist well prepared for its daily clinical practice, in order to choose the best preventive and therapeutic procedures for each patient. This review article has the objective to characterize and integrate the biochemical basis of dental aging and therefore, to develop the understanding of the basic mechanisms that lead to the changes that accompany this condition.

Materials and Methods

In the present review article, the bibliographic research was made exclusively through the Pubmed and the Faculty library. The articles selected were all in the English language. Only free access articles were used and, also, articles that had no relation with the theme were excluded. No specific statistic tests were used, only statistics that was part of the investigation articles. The keywords used for research were: aging, oral biochemistry, teeth structure, teeth composition, enamel proteins and dentin proteins.

Teeth Composition and Structure

Teeth are formed by three mineralized tissues: enamel, dentin and cement; and another, that in normal/healthy conditions is non-mineralized, the pulp.[5]

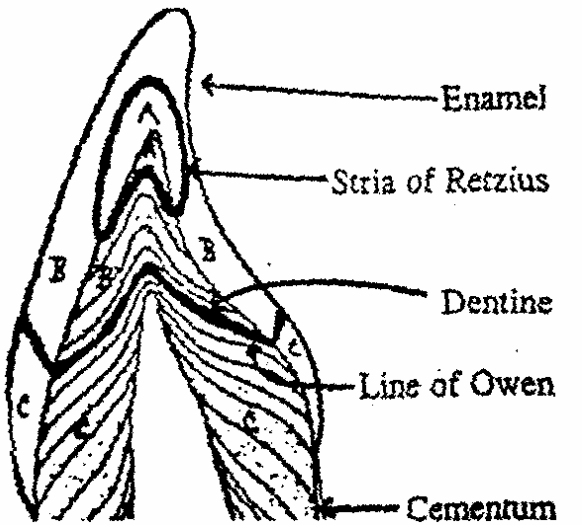

Fig.1 - **Longitudinal cross section of a deciduous incisor tooth showing enamel, dentine and cementum.** Zones A, B, and C in enamel and dentine represent the earlier to later calcifying portions of the tooth crown. The pattern of formation is reflected in contour lines of Owen in dentine and stria of Retzius in enamel. The accentuated growth line between zone A and B is the approximate division between the second and third prenatal trimester, and the line between zones B and C represents the approximate location of the birth line. Acid dissolution may sample zones of "dome" enamel whereas laser ablation samples finer areas of enamel and dentine.[5]

Enamel forms the exterior layer of the crowns of human teeth; dentine comprises the interior of the crown and roots, and a thin layer of cementum covers the roots. In addition to the “primary” cementum and dentine, which are formed during early in life, secondary (circumpulpal) dentine and secondary cementum are continuously deposited.[5]

Enamel Biochemical Composition

Enamel is composed by about 95% mineral by weight, but 87% by volume.[3,6,7] This inorganic material comprises mostly calcium and phosphate ions, which appear as a crystalline structure in the form of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$.[6,7]

Besides calcium and phosphate, the most important chemical components are magnesium and carbonates. Other trace elements found in enamel are sodium, potassium, zinc, lead, strontium, iron and fluorine. These minerals are incorporated into the enamel by ion exchange or adsorption at the crystal surface, inside or in the hydration layer between the crystals.[6]

Some mineral elements like fluorine, lead, zinc and strontium, are present at higher concentrations on the external surface of enamel while carbonate, sodium and magnesium are mainly present in the inner layers, which are those closest to the enamel-dentin junction. The presence of carbonate in the hydroxyapatite connectivity networks disrupts the structure of these networks.[6] It acts replacing the phosphate, which reduces the size of the network. If the exchange comprises hydroxyl ions, the network will expand.[6] Globally, the greater the amount of carbonate present in the enamel, more easily minerals are dissolved in the presence of acids.

The large amount of mineral in enamel accounts not only for its strength but also for its brittleness.[8] Dentin, which is less mineralized and less brittle, compensates the rigid characteristics of enamel and is necessary as a support. On radiographs, the differences in the mineralization of different portions of the tooth can be noted; enamel appears more radiopaque (or lighter) than both dentin and pulp, since it is denser, both of which appear more radiolucent (or darker).[9]

The normal color of enamel varies from light yellow to grayish white. At the edges of teeth, where there is no dentin underlying the enamel, the color sometimes has a slightly blue tone. Since enamel is semitranslucent, the color of dentin and any material underneath the enamel strongly affects the appearance of a tooth. The enamel on primary teeth has a more opaque crystalline form and, thus, appears whiter than on permanent teeth.[8]

Enamel does not contain collagen, as found in other hard tissues such as dentin and bone, but it does contain two unique classes of proteins - amelogenins and enamelins.[8] In its first formed state, it contains approximately 90% of the former and 10% of the latter.[6,10] While the role of these proteins is not fully

understood, it is believed that they aid in the development of enamel by serving as a framework for minerals deposits to form on, among other functions.[8]

The amelogenins are lost preferentially during the process of maturation when the matrix is removed and replaced by the growth of the apatite crystals. The amelogenins are rich in proline, leucine, histidine and glutamine, but unlike collagen or keratin they contain no hydroxyproline. One of the smallest amelogenins (with a molecular mass around 5 kDa) is rich in tyrosine and is therefore known as TREP (tyrosine-rich enamel peptide).[6]

The enamelins, however, have a molecular mass between 50 and 70 kDa. They contain less proline, glutamine and histidine but more asparte, serine, glycine, alanine and arginine.[6] They are usually glycosylated with about 4% hexosamine and 4% sialic acid. Recent work suggests that there is one enamelin protein that is identical to, or at least very similar to, serum albumin.[6] It remains in enamel after maturation, appearing to be bound to the enamel crystals, where it is located in its long axis, perpendicular to the length or c-axis.[6] It is possible that the enamelins play some effective role in determining the shape and size of enamel crystals. Mature enamel contains acidic proteins, low molecular weight lipids, and some carbohydrate and organic acids such as citrate and lactate.[6]

Nevertheless, at this phase enamel has only low amounts of the softer organic matter.

Enamel is also avascular and has no nerve supply within it. In addition, it is not renewed; however, it is not a static tissue as it can undergo mineralization changes.[9]

Dentin Biochemical Composition

Sandwiched between the hard exterior cap of enamel and the central tooth chamber, dentin is the major structural component of the tooth.[11] It is composed by approximately 70% mineral, 20% organic matrix and 10% water by weight, and 45%, 33% and 22% by volume, respectively. Between all the natural changes that occur in the human teeth with aging, perhaps the most notable are the changes in physical and chemical structure of dentin.[2] Dentin seems particularly suitable for the study of biochemical changes in teeth with aging because it is composed of approximately 20% organic matrix (mainly collagen) and is thought to have minimal collagen turnover. As it happens in bone tissue, dentin collagen constitutes almost 90% of the organic matrix,

and is primarily from type I.[4] Collagen molecules and their crosslinks are biochemically important in mineralized tissues, providing plastic, ductile properties, whereas the mineral component confers stiffness. The content of crosslinks in human dentin also changes with age.[4]

Softer than enamel, this hydrated biological composite structure is composed of intertubular and peritubular matrices, and dentinal tubules.

Intertubular dentine consists of a fibrous network of collagen with deposited mineral crystals. Peritubular dentine is a more highly mineralized tissue with fewer collagen fibers than intertubular dentine.[12] In fact, it forms a highly mineralized shealt lining each dentinal tubule. Dentinal tubules contain odontoblasts and extend from the pulp to the tooth's outer edge.[12] Odontoblasts are highly differentiated post-mitotic cells that are capable of secreting primary, secondary and tertiary dentin matrix.

With their peripheral location in the dental pulp and their cellular processes into dentine, the odontoblasts have been demonstrated to detect and respond to dentine injury.[13]

They arise from undifferentiated pulp cells; they lay down dentin matrix, assist in its calcification and maintain the vitality of the tissue.[3]

Dentin matrix can be classified as either primary, secondary or tertiary in origin according to the chronology and circumstances of its secretion by the odontoblasts.[13] Primary dentin is secreted during tooth development, until the completion of root formation. Physiological secondary dentin is laid down after completion of root formation throughout life. Unlike primary or secondary dentin that forms along the entire pulp-dentin border, tertiary dentin is focally secreted by odontoblasts in response to primary and secondary dentin injury.[13] If these cells survive the injury, they may secrete a reactionary dentin matrix of repair. On the other side, if they are irreversibly injured, they must be replaced by a second generation of newly differentiated odontoblast-like cells, before the secretion of a reparative dentin matrix of repair can take place.[14]

The mineral phase in dentin mainly consists of carbonate-substituted hidroxylapatite ($Ca_{10}(PO_4)_{6-x}(OH)_{2-y})(CO_3)_{x+y}$, (where $0 \leq x \leq 6$, $0 \leq y \leq 2$) in the form of small plates.[15] As referred previously, the organic phase contains about 90% fibrous proteins (primarily type I collagen), whilst the rest of the organic phase is composed by lipids and non-collagenous matrix proteins.[8] These proteins can be divided in different groups: the Gla (y-carboxyglutamic acid) osteocalcin like proteins (a protein found in bone); the phosphoproteins, in which phosphoserine and aspartic acid account for about 80% of the amino acid composition; the matrix Gla proteins; and the

proteoglycans which have not been rigorously characterized yet. In this group are also included several acidic glycoproteins and some seric proteins.[6, 16]

Osteocalcin is a vitamin K-dependent protein. It is synthesized by osteoblasts and odontoblasts, and is a well-known marker of viability, differentiation and osteogenic ability in those cells.[17]

The highly phophorylated phosphoproteins are thought to be unique to dentin, and the distribution of the proteoglycans is not the same as that in bone. Many of these non-collagenous proteins have been shown to promote apatite crystallization, whilst some proteoglycans inhibit it. Although the detailed roles of them are far from being elucidated, it is likely that they are important in controlling the growth and orientation of apatite crystals.[3]

Other organic materials in dentin include lipids (about 0,1%) and citrate (about 0,9%).[3]

Phosphoproteins in dentin

More than half of the total amount of phosphoproteins in dentin corresponds to the so-called highly phosphorylated phosphoprotein or phosphophoryn. It contains more than 20% phosphate, probably as phosphoserine, since serine makes up about 50% of its amino acid residues. Another acidic amino acid, aspartic acid accounts for a further 40% of total residues; it is not surprising, therefore, that phophophoryn is one of the most acidic phosphoproteins known, with an isoelectric of 1.1.[3] There is evidence that some of the highly phosphorylated phosphoprotein molecules are linked to collagen and this may have some significance in the mineralization process.[3]

The properties of the highly phosphorylated phosphoprotein suggest that it may be significant in the mineralization process since it binds calcium ions strongly and it can induce hydroxyapatite formation when present in low concentrations and stabilized on some solid support.[3]

The other phosphoproteins of dentin are much less phosphorylated and they resemble more likely those found in bone.

Proteoglycans in dentin

The proteoglycans form the second major group of non-collagenous macromolecules in dentin.[3]

Proteoglycans are carbohydrate-rich polyanions with a high molecular weight constituted by a polypeptide core to which is attached one or more glycosaminoglycans, i.e. repeating disaccharide units with sulphate ester groups linked at position 4 or 6.[18] The presence of chondroitin 4–6 sulphate is very well described on predentin, dentin and cement and it is claimed to regulate the biophysical properties of dentin proteoglycans, which in turn may regulate the final collagen fibrils three-dimensional arrangement. In other words proteoglycans may account for the three-dimensional appearance of the dentin organic matrix due to their ability to fill space, bind and organize water molecules, and repel negatively charged molecules.[18]

Pulp Composition

A unique feature of dentin is that it is the mineralized tissue that surrounds the pulp, an unmineralized tissue. Dental pulp not only functions to provide nutritional and sensory properties to dentin, but also has its own reparative capacity[19]

The dental pulp is a loose connective tissue contained within a central cavity surrounded by an avascular hard tissue case. Neverthless, the pulp receives a rich vascular supply. The blood vessels enter the pulp space via the apical foramen in association with the sensory and sympathetic nerve bundles.[20]

Its extracellular matrix is mainly made up of proteoglycans. The glycosaminoglycan parts of these molecules are long-chain carbohydrate polymers that retain water to form a gel. In many tissues the function of such gel is to resist compression, but in fully formed tooth where the pulpal cells and structures are protected by the surrounding dentin its role is more that of space filling.[3]

The pulp contains around 88% water, a proportion high in comparison with other tissues. The principal glycosaminoglycan of the pulp is chondroitin 4-sulphate (estimated as about 55% of the total weight). Chondroitin 4-sulphate binds calcium readily; thus, it helps to maintain a high calcium level in the pulp, providing a store of calcium to assist in the mineralization of the dentin matrix.[3] Next in concentration is keratin 1-sulphate (28%), most of which is linked to protein molecules. The only other

glycosaminoglycan present in a significant amount is dermatan sulphate (13%), a substance which seems to be important in collagen fibrillogenesis.[3] There is a small amount of hyaluronan, but virtually no heparin sulphate or chondroitin 6-sulphate.

The fibrous component of the pulp is almost entirely collagen. Elastin is present only in the walls of the blood vessels. However, there are some glycoprotein fibrils also present, similar to those which surround elastin fibers; these constitute the oxytalan fibers.[3] Collagen makes about one-third of the dry weight of the pulp. The types normally present are types III, I and VI in order of their relative amounts. A very small amount of type V is also found. The normal function of these collagen molecules is to confer some rigidity to a tissue; in the pulp that probably means maintenance of position of the vessels, nerves and cells. Collagen type III is usually associated with some degree of elasticity or extensibility of the tissue.[3]

Other proteins found in the pulp include the glycoproteins fibronectin and tenascin. Fibronectin binds cells to collagen (particularly type III) and to the sulphated glycoproteins of the matrix; it is probably important in stabilizing the special arrangement of pulp cells and matrix fibers.[3] It disappears from the mesenchyme when odontoblast differentiation begins and has therefore been suggested to play some role in this process. However, a more likely candidate for an organizer molecule is tenascin, which is produced by the cells of dental papilla but not by odontoblasts and has been shown to be widely distributed in dental pulps which retain the capacity to produce calcified tissue.[3]

A number of enzymes have been detected and assayed in dental pulp. These include the enzymes of the glycolytic pathway, as well as alkaline phosphatase (ALPase), which is associated with the odontoblasts and the predentin layer.[3] ALPase activity in pulp is as high as that in bone and is thought to be essential for biomineralization. This enzyme activity in odontoblasts and subodontoblasts cells is higher than in undifferentiated mesenchymal cells. Furthermore, ALPase, as a pulp-capping agent, stimulates pulp tissue to form the dentin matrix.[21]

Collagenase has also been detected in pulp and is thought to be secreted by the odontoblasts. It is normally inactive because of the presence of inhibitors.[3]

Among the pulp matrix are located the different cell types described above and nutrients circulate there through ranging from blood vessels into cells as the products of the latter follow the opposite path. Changes in the composition of this matrix, caused by age or some pathological change, interfere with the functionality of the pulp, causing metabolic changes, diminishing cell function and leading to irregularities in the deposition of minerals.[6]

Cement Composition

Cementum (or cement) is a thin layer that covers the roots of teeth. It is relatively similar to bone in a number of characteristics, including embryological origin, basic structure and degree of calcification.[5] Cement has an inorganic content very similar to bone[3,6], being composed mainly by calcium and phosphorus, but also containing magnesium, fluoride and other mineral traces.[6] Its mineral content is around 65% of weight. The organic matrix represents about 23%, corresponding the 12% left to water.[3,6] Its apatite crystals are similar in size and structure to bone and dentin[5] and, as it happens in dentin, there is a relatively high proportion of carbonate and magnesium substitution in the crystal lattice.[3]

The organic matrix also seems to have similar features to the ones observed in bone and dentin. It appears as a base substance or fundamental matrix on which are distributed the collagen fibers. It is on this matrix that the calcium salts are deposited. Approximately 90% of the proteins present in the organic matrix are collagen[6], mainly type I[3, 6], but is also present some collagen type III, which comes to represent between 5% and 7% of total collagen molecules.

Among the non-collagenous proteins of the organic matrix, it must be emphasized the presence of a phosphoprotein, very similar to that found in bone. It is a glycosylated sialoprotein, which is known as osteopontin. (espanhol) In the layer next to mantle dentine is a higher concentration of non-collagenous proteins, particularly the osteopontin. This protein is thought to bind mineral, type I collagen and osteocalcin; it can also cross-link to fibronectin. This suggests that it has an important function in the binding of cementum to dentin and in tissue cohesion. It is found at the cementum lines and bone cement lines where a new layer of hard tissue has been laid down on an existing hard tissue surface.[3] Other organic molecules in cementum include fibronectin, tenascin, cementum-derived attachment protein and a number of growth factors.

The cementum matrix laid down next to dentin contains collagen fibers, amelogenin-like proteins, osteopontin, bone sialoprotein, osteocalcin and alpha-2 HS-glycoprotein. Bone acidic glycoprotein is not found in this cementum matrix.[3]

The base substance or fundamental matrix consists of sulfated or non-sulfated proteoglycans and neutral and acid mucopolysaccharides. The cement also contains sialic acids. In humans, glycosaminoglycans have been identified as part of the proteoglycans. 16% is hyaluronic acid, 53% chondroitin sulfate and 31% dermatan sulfate.[6]

One notable feature of cementum is that in addition to a primary layer in mammals, it is continuously deposited in annual rings, which has been used in wildlife biology and bioarchaeology as a method for determining age at death.[5] Because of its continued deposition, cementum chemistry provides a means of tracking annual life history changes until death. Outridge et. al (1996) have shown that lead varies by cementum layers[5]

The layered appearance is due to structural differences in the mineral phase, an optical phenomenon that is possibly related to altered mineral crystal orientations[22] and reflects a cyclic annual formation pattern. One pair of alternating light and dark lines should therefore correspond to one year in an individual's life.

Age related changes in Enamel

Enamel does not undergo any further mineral deposition after it has been laid down by the ameloblasts. Its surface can, however, be modified grossly by abrasion, attrition, erosion or dental caries, and at a crystal level by ion exchange, demineralization and remineralization.[3] When a tooth erupts into the mouth it has a surface layer which is bigger in fluoride concentration than are deeper layers. As the tooth ages it takes up fluoride from saliva or ingested water and food so that the surface layer should steadily increase in fluoride concentration.[3] However, surface enamel is lost by erosion, attrition and abrasion so that the actual enamel surface in an older individual may no longer be so high in fluoride concentration. Other ions exchange with the surface apatite so that concentration gradients are produced through the enamel.[3]

Irrespective of the common sense that enamel seems less affected by the effect of age, Park et al. showed that superficial enamel gets harder with age. This fact can be understand as a direct result of enamel mineralization with age, since this process occurs at the outermost surface.[23]

Several authors have reported that teeth tend to darken with age[24] and may become more liable to cracking. The color changes may be due to progressive staining (although no obvious chemical differences have been detected which might explain the color) or to some physical change in enamel rendering the underlying dentin more visible.[3] One explanation of the tendency to crack has been that the outer layers of dentin may lose some water and start to shrink away from the enamel so that it loses its 3D support. The most significant experimental observation on older enamel is that it is less permeable to dyes and radioactive isotopes than at an earlier stage.[3] This

suggests changes in composition in both the mineral and organic phases of the enamel. It has been suggested that possibly that some small increase in calcification occurs during aging; this would explain loss of permeability, and possibly make the enamel more translucent and also more brittle. However, small increases in the degree of calcification would be difficult to detect in such a highly calcified tissue.[3]

Several studies have revealed that the aging process may affect enamel physical properties.[23, 25, 26] Considering that this process may influence the mineral concentrations of dental enamel, the mineral densities between young and old age groups were compared by He, Bing and colleagues.[27] They have found differences in the mineral density in the outer-layer enamel between the young and old age groups. Abundant mineral ions and fluoride in the saliva, as well as prolonged exposure to the oral environment by the outer enamel layer in the old age group may promote the replacement of the enamel matrix by fluoroapatites, ultimately leading to an increase on tissue density and a decreased permeability.[28, 29] In the middle and inner enamel layers, the observed differences between the two age groups were not statistically significant.[27]

Age-related changes in Dentin

The properties and the microstructure of young healthy dentin are now well understood and can be used as a baseline for characterizing how dentin ages.

A team of scientists from the University of California at San Francisco (UCSF) has observed that dentin becomes brittle with age, referring that embrittlement appears to occur as a natural consequence of the aging process.[9] Although the cause of this embrittlement is still unknown, recent evidence suggests that age-related changes at the molecular level may be responsible for it.[9]

The main age-related change in older teeth includes a gradual enlargement of the peritubular dentin and intratubular mineral deposits, which often result in narrowed or completely occluded tubules. This results in a significant decrease in dentin permeability.[30]

Dentin differs from enamel because it can be formed continuously throughout life. As it is penetrated by the dentinal tubules which contain cell processes or dentinal fluid, the whole thickness of dentin is in communication with fluids within the body until those tubules become obliterated. As teeth age, the tubules begin to fill with mineral

deposits, starting at the root end and working upward. This phenomenon is known as transparency because the mineral deposits prevent the tubules from scattering light.[9]

In circumpulpar dentin the collagen fibers of the matrix are arranged in layers which are at right-angles to the odontoblast layer that has laid them down. This contrasts with the thin layer of mantle dentin immediately next to enamel where the fiber directions are parallel with the tubules and with the hyaline layer next to the cement where a similar situation exists.[3]

Regular secondary dentin is a continuation of the circumpulpar dentin which is laid down throughout life. It is usually demarcated from the circumpulpar dentin by an accentuated incremental line where a change in direction of the dentinal tubules occurs.

As dentin ages there is deposition of a layer of more highly calcified material on the walls of the tubules. This material appears finely granular, contains collagen fibrils only as inclusions, and may present amorphous calcium phosphate rather than apatite. It is known as peritubular dentin.[3] In sclerotic dentin the tubules are completely occluded by the further deposition of calcified material, similar in appearance to the peritubular ones, although mineral deposits have been identified as mainly apatite. Sclerotic dentin appears translucent when viewed in ground sections in water.[3]

The appearance of sclerotic dentin is related to attrition but another type of translucent dentin is produced in the normal course of aging of dentin. It is again produced by the deposition of apatite into the dentinal tubules, the process in this instance beginning in the root and progressing coronally. The extent of the translucent area is directly related to the age of the tooth and hence can be used as a guide to age estimation in forensic dentistry.[3]

As referred above, with aging, normal dentin is altered to form what is known as “transparent” (or sclerotic) dentin. The tubules gradually fill up with a mineral phase over time, beginning at the apical end of the root and often extending into the coronal dentin, possibly due to a passive chemical precipitation process, decreasing the amount of light scattered off the lumens.[34]

Transparency is a common age-induced pathology in human dentin and has been associated with a definitive reduction in the fracture resistance.[35]

Porter and colleagues examined the effect of aging on the mineral phase of dentin via high-resolution transmission electron microscopy. It was found that the mineral crystallites are smaller in transparent dentin and the tubule lumen appeared to be filled with coarse minerals made of hydroxyapatite.[34] Significant differences in nanostructure between intra- and inter-tubular dentin in transparent teeth were

observed. Although the nature of the age-related change is not yet known, the authors suggested a “dissolution-reprecipitation” mechanism for the formation of transparent dentin.[34]

Another important feature relies on the slower formation of dentin throughout life, which allows it to incorporate fluoride from the plasma via pulpal tissue fluid to a greater extent.

Hence the pulpal surface of dentin shows the highest concentrations of fluoride and this usually exceeds those in surface enamel in older subjects.[3]

The content of crosslinks in human dentin also changes with age; in fact, recent studies have detected a linear increase in deoxypyridinoline content (a nonreducible trifunctional crosslink) in relation to the individual’s age.[31]

Walters C. and Eyre D.R also found in earlier studies that in human dentin, hydroxypyridinium crosslinks increased with age and became the predominant crosslinks as the two reducible residues, dehydrodihydroxylysinonorleucine and dehydrohydroxylysinonorleucine, diminished.[32]

Matrix metalloproteinases (MMPs) are a family of enzymes which globally are able to degrade collagen in a variety of tissues, including dental tissues, under diverse physiological and pathologic situations.[33] The role of matrix metalloproteinases in mineralized dental tissues, appears to be played in the organized processing of the enamel protein matrix during enamel formation.

Gelatinase A (MMP-2), in latent and activated forms, was first detected in relatively constant proportions throughout the developing enamel. Moreover, cultured human odontoblasts derived from adult teeth have recently been shown to synthesize gelatinase A.[4]

Murray *et al.* studied the age-related odontometric changes of human teeth and found that the changes taking place in teeth with aging included increased dentinal thickness and decreased cellularity. Except for the incisor fibroblasts, the reduction in density of each pulp cell type was greatest in the root aspect of all tooth types.[36]

Finally, some observations demonstrated a decreased thickness of the remaining occlusal tertiary dentin indicating that the adaptive response might decrease with age.[37]

Age-related changes in Odontoblasts

The number of odontoblasts has been reported to fluctuate from birth to 23 years of age, after which they start to decrease progressively to approximately half their number until 70 years of age is reached.[14]

Furthermore, in some studies the quantity of reactionary dentin secreted by each odontoblast in permanent premolar teeth appeared to increase as the age of the patient increased.[14]

The dentinogenic response potential of teeth could be expected to reach a maturation plateau and thereafter diminish into old age, as a reduction in the rate of dentinogenesis has been observed in the elderly.[14] Moreover, age has been observed to be an important modulator in the secretion of reactionary, physiologic sclerotic and reparative dentin.[14] These changes in odontoblasts activity can correspond to changes observed at the molecular level with the aging process. Indeed, a reduction in length and cytoplasmic organelles with aging was observed, which is in accord with a decreased synthetic and secretory capacity of these cells.[38]

Lovschall et al. refer that in general, aged odontoblasts in the coronal pulp chamber were substantially taller than those in the roots. The varied size and shape of odontoblasts probably reflect regional changes in their functional activity.[37] Odontoblasts were also found to develop an autophagic-lysosomal system organized mainly by large autophagic vacuoles that are acid-phosphatase-positive to various degrees. Autophagic activity has been described as a fundamental survival mechanism for long-lived post-mitotic cells and is thought to be associated with the aging process.[39] However, progressive changes in lysosomal function and a reduction of autophagic activity have been related to the accumulation of lipofuscin, a process that mainly affects long-lived cells. Lipofuscin results from a slowly progressive accumulation of waste products that affects mainly post-mitotic cells, reducing cell viability. In general, the accumulation of lipofuscin within lysossomal compartments in long-lived cells results in a harmful influence on these cells, decreasing lysossomal activity against oxidative stress and reducing autophagic turnover.[40] Therefore, the detrimental effects of lipofuscinogenesis in human ododntoblasts as a natural consequence of aging might cause a reduction in dentinogenic activity and cell viability.[39]

Another notable age-related change is that odontoblast layer loses its continuity in addition to losing the subodontoblastic cell-rich zone in the pulp floor. Since the

odontoblast-lineage cells are supposed to reside in the subodontoblastic layer, the loss of odontoblasts is attributed to the lack of a progenitor cell pool.[41]

As mentioned above, with age, there is a reduction in the number of odontoblasts and pulp cells[42] and the secretory activity of these cells is reduced but this may be re-activated after injury.[19]

Age-related changes in Pulp

Little is known about the effect of aging on the characteristics and functions of pulp cells.

Morphological and histological studies have shown that pulp volume reduces with aging, and this process is accompanied by fibrosis, calcification, arteriosclerotic changes, decreased number of blood vessels, reticular atrophy, loss of cellularity, and odontoblast degeneration.[20, 43, 44]

These changes are considered to be closely associated with alterations in pulp hemodynamics.[44] Reduction of chamber size is a common phenomenon in aged teeth[45] and it frequently leads to difficulty in clinical treatment of the pulp.

Once tooth formation is complete, the pulp is totally surrounded by a mineralized environment, and because of the continuous slow secretion of physiological secondary dentin, the space occupied by the pulp is gradually reduced. The number of calcifications (pulp stones and diffuse calcifications) increases with advancing age.[19, 36, 46]

Muramatsu et al. showed results of a distinct decline of osteocalcin mRNA in aged human pulp. These results suggest that reduction of osteocalcin may be related to the reduction of chamber size and should be considered a characteristic of aged pulp tissues.[16]

Change in size is also associated with a decrease in blood supply as the apical foramen is almost obliterated by secondary dentin and cement.[3]

Ikawa *et al.* acknowledged in their studies that the reduction in Pulpal Blood Flow (PBF) induced by cold stimulation was significantly decreased with increased age. This is considered to be associated with the histological findings of age related arteriosclerotic changes in tooth pulp.[44] The resting PBF during control recording was also decreased with age, which is in agreement with the histological findings; that is, the number of blood vessels was decreased and the size and volume of the pulp was also reduced due to the increase in calcified tissue with age.[44]

The number of cells in the pulp decreases with age in terms both of fibroblasts and of odontoblasts.[3] The odontoblasts develop the so-called wheatsheaf appearance as intercellular and intracellular vacuoles appear. Calcified masses known as pulp stones are more frequently observed with age. They arise by calcification of collagen fibers, although some have a more dentin-like morphology with irregular tubules. It has been suggested that calcification is more likely to occur where collagen fibers have a greater extent of cross-linking such as what occurs in aging.[3]

Despite there are some reports that claim that there is an increased amount of collagen in the aged dental pulp, it is thought that the actual numbers of fibers is decreased. The aggregation of small fibrils into larger coarse fibrils in the smaller volume gives a false impression of a relative increase in collagen. The rate of synthesis of collagen decreases and the existing fibers become more cross-linked. The major cross-link in human pulp collagen is dihydroxylysinonorleucine.[3] The relative amount of this cross-link increases up to the age of 40 years but decreases thereafter as other links are formed.[3]

Biochemical investigations of human teeth have revealed a decrease in collagen concentration, which occurs at about the same time as eruption and root closure. However, for the rest of the life of the tooth, there is no change in collagen content. Hillmann G. and Geurtsen W. suggested that all the pulp collagen (types I, III, V and VI), remains in the pulp during aging but they become compressed into a smaller volume as the cavity shrinks during dentinogenesis.[46]

Little is known about the life span of pulp fibroblasts. However, a decline in the total number of cells has been reported in the aging human pulp.[19]

A significant decrease in the ammount of sub-odontoblast cells with aging was observed in the human pulp.[42]

The amount of fibrous components in the pulp matrix apparently increases with age, particularly in the radicular pulp.[37] The narrowing of the pulp chamber due to dentin formation apparently facilitates the accumulation of collagen fibers. The decreasing diameter of the root canal can promote an increase in the relative volume of the remaining nerves, vessels and fibrous components, corresponding to a decreased cell density.[37]

In a dynamic interplay of cell proliferation and cell death, the developing tooth retains precisely the type and number of cells needed to proceed into maturity. It is admitted that odontoblasts are terminally differentiated cells that survive as long as the integrity of the tooth is preserved. However, the dental pulp volume decreases gradually on aging due to the continuous production of dentin matrix by odontoblasts.

This age-related pulp chamber reduction is associated with the elimination of a certain number of odontoblasts by apoptosis.[47]

Also, ALPase activity decreases with in vivo aging in human pulp cells. When a odontoblast layer is destroyed due to caries or operative dental treatment, surrounding pulp cells switch their functions and begin to proliferate after which, pulp cells differentiate into odontoblasts to form reparative dentin. Therefore, the decrease in proliferative ability and ALPase activity in pulp cells with aging may result in deficient formation of reparative dentin formation.[20]

Although cell viability deteriorates in aged tissues in general, these findings imply that aged dental pulp still has the ability to create dentin but at a diminished rate.[16]

Kawagishi and partners concluded in their study about pulpal responses to cavity preparation in aged rat molars that the aged pulp tissue possesses the same rapid rate of recovery as that in young adult teeth.[41]

Putting it all together, changes in the dental pulp in older individuals result from the progressive reduction in pulp size as a secondary dentin advances into the pulp space, from changes in the fibers of its connective tissue, and from changes in blood supply and innervations.

As the repair capacity of the dentinal pulp complex appears to be age dependent; this may explain differences in success of restorative treatments between patients.[36]

Age-related changes in Cement

Cement is laid down throughout life and shows incremental growth lines which can help in determining the age of the individual from whom the tooth was removed.[3] With age this tissue becomes less permeable to dye molecules and ions. This may explain why the deeper layers of cement do not have viable cementocytes in them: nutritive molecules may not be able to reach those regions. Like dentin, cement continues to take up fluoride where its thickness increases slowly throughout life, and relatively high concentrations may therefore be present at the surface adjacent to the periodontal ligament.[3]

As the tooth ages the cement becomes progressively less permeable from the dentinal side although it remains permeable from the periodontal ligament side. The amount of cement towards the apex of a tooth root increases throughout life.[3]

Ababreh KT et al. studies have attempted to elucidate differences in expression of proteoglycans between clinical categories or age groups. The results obtained reflected quantitative changes in the proteoglycans of cement and/or alterations of proteoglycans structure, as aging and diseased cells are likely to synthesize decreased quantities or even altered forms of these macromolecules in terms of glycosaminoglycans chain length, sulphation and species, rather than completely cease protein synthesis.[48]

Conclusion

Aging requires multiple adaptation of physiological processes, affecting differentially all the tissues of the organism. Oral tissues and, particularly, teeth, are no exception. In fact, there are important changes on the different components of teeth that occur with aging, affecting the enamel, dentin, pulp and cement structure and/or functions.

Ultimately age-related changes in teeth are based on biological markers of age.

An understanding of the corresponding changes to the underlying ultrastructure of teeth is important from the perspective of evaluating age-related changes in the mineralization and could assist in the design of measures to counteract their deleterious effects.

Changes in teeth due to aging and disease processes introduce clinically significant alterations in tissues structure and properties, and our understanding of these alterations is only in the beginning. The impact of these alterations on current and future treatments is expected to be profound.

Understanding the disadvantages of age-related changes such as, reductions in the pulpal cell population, and using the advantages such as increases in dentinal thickness, will benefit the provision of restorative care for the worldwide increasing numbers of older aged patients.

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