Comparison Between The Effects of Different Mouthwashes on Enamel, Cementum And Dorsal Surface of The Tongue of Albino Rat (Histological and Scanning Electron Microscopic Study)

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INTRODUCTION

Mouthwashes are commonly used are liquid preparations for being applied on teeth and mucosa of the oral cavity and pharynx in order to exert an antiseptic, astringent and sedative local action. Various types of mouthwashes are available in the market, they differ in content and as such are tolerated to specific requirements. Normally, the active ingredients of mouthwashes are antimicrobial agents which have a temporal effect of reducing the totality of the microorganisms of the oral cavity ***(Otomo, 1990; Farah et al., 2009).***

Although mouthwash use is considered an adjunctive procedure in oral prophylaxis, it has been associated with oral pain and mucosal dryness especially when alcohol containing mouthwashes are used**.** Pain during mouth rinsing can be discouraging and detrimental to patient compliance and may hinder optimal mouthwash rinsing ***(Siddappa, 2002; Lemos and Villoria, 2008)***.

Mouthwashes may potentially cause immediate or late effects on the surfaces they are applied to such as enamel, causing demineralization, dyeing and dental erosion injurie ***(Sissons et al., 1996)***. The most common side effect of mouthwashes is hypersensitivity stomatitis manifested by erythema, ulceration, or epithelial sloughing ***(Eldridge el al., 1998).*** It is matter of debate if mouthwashes could be considered as a risk factor in cancer development. (***McCullough and Farah, 2003; Cole et al., 2003)*****.**

REVIEW OF LITERATURE

Enamel

Enamel is one of the first biological tissues to be examined using the light microscope. Enamel layer covers the crown of the tooth and is the hardest substance in the body ***(Boyde, 1989; Cole and Eastoe, 1988).*** Enamel is composed of both an organic and an inorganic phase; the organic phase is composed mainly of proteins such as amelogenin, ameloblastin and tuftelin. The enamel inorganic phase is composed of well-packed nanocrystals made of calcium phosphate hydroxy-apatite crystals (HA) with small amounts of incorporated trace elements (***Nanci, 2003***).

On the other hand, the basic structural unit of enamel is the enamel prism or rod, rod sheaths and interred regions. In cross section, the shape of enamel prism approximates to one of the three main patterns: in pattern I enamel the prism are circular; in pattern II enamel the prisms are aligned in parallel rows. While in pattern III enamel the prisms are arranged in staggered rows such that the tail of a prism lies between two heads in the next row, giving the keyhole appearance. Enamel rods measure about 5 um in breadth and 9um in height over the extreme head to tail dimensions ***(Berkovitz et al., 2002).***

In ground sections of enamel, the most prominent layering is marked out by dark bands known as the brown striae of Retzius

(***Boyde, 1989).*** They represent a regular growth rhythm which is regular enough to use as the basis of crown growth studies (***Dean, 1987)***. The top of each enamel layer open out and the most occlusal edge of each layer becomes visible at the surface. In each layer, this edge is laid down slightly cervical to the preceding edge, giving rise to the pattern of crests and grooves (perikymata or imbrication lines) seen at the surface ***(Risnes, 1985).***

Another surface structure is the enamel rod ends which are concave and vary in depth and shape .They are shallowest in the cervical regions of the surfaces and deepest near the occlusal or incisal edges. In addition, cracks originally were used to describe the narrow fissure like structures that are seen on almost all surfaces. It has since been demonstrated that they are actually the outer edges of lamellae and they extend for varying distances along the surface at right angle to dentino-enamel junction and most of them are less than a millimeter in length, but some are longer ***(Bhashkar, 1991)***.

The outer most layer of enamel is prismless enamel. The thickness of this layer is approximately 30 um and it has been described in all deciduous teeth and in 70% of permanent teeth. This prismless enamel is found least often over the cusp tips and most commonly toward the cervical areas of the enamel surface. In this surface layer no prism outlines are visible , and all the apatite crystals are parallel to one another. It is also somewhat more heavily mineralized than the bulk of enamel beneath it (***Nanci, 2003*)**.

Cementum

Dental cementum is unique in various aspects, it is avascular and not innervated, does not undergo continuous remodeling like bone, but continues to grow in thickness throughout life (***Bosshardt and Selvig, 1997)***.

Traditionally, cementum has been classified as cellular and acellular, depending on the presence or absence of cementocytes in its structure. Acellular cementum covering the cervical portion of the root is critical for tooth attachment to the adjacent periodontal ligament (PDL), while cellular cementum covering the apical root is hypothesized to play a role in post-eruptive tooth movement and adaptation to occlusion. Mixed stratified cementum includes layers of both acellular and cellular types, and is present on apical roots or in the furcation region of some species ***(Bhashkar, 1991)***. Another classification includes intrinsic or extrinsic fiber cementum, depending on the presence of collagen fibers formed by cementoblasts or by fibroblasts, respectively ***(Schroeder, 1986)***.

To about equal parts per volume, cementum is composed of water, organic matrix and mineral and about 50% of the dry mass is inorganic, and consists of hydroxyapatite crystals. The remaining organic matrix contains largely collagens, glycoproteins and proteoglycans ***(Bosshardt and Selvig, 1997).*** Cementum has adaptive and reparative functions, playing a crucial role to maintain occlusal relationship and to protect the integrity of the root surface ***(Gongcalves et al., 2005)***.

Cementum is found along the tooth root and primarily serves to hold the tooth in place by binding collagen fibers (Sharpey's fibers) that are continuous with the principal fibers of the periodontal ligament. These fibers are orientated more or less perpendicularly to the cementum surface and play a major role in tooth anchorage ***(Nanci,2003)***

Rats are considered to be good experimental models because the periodontal anatomy of a rat molar is very similar to that of a human**.** Additionally the genetic, clinical radiographic and histological aspects of the rat periodontium are similar to the human periodontium. The similarities published to date include the cementum structure (***Klausen, 1991).*** The root formation in rats is 17–20 times faster than in humans. This implies that 9- to 12-month-old rat specimens when compared to 40- to 55-year-old humans could be significantly more mineralized (***Bosshardt and Schroeder, 1996).***

In comparison, rat cementum had a significantly lower decrease in elastic modulus when hydrated than human cementum. The significant differences in percent reduction in elastic modulus under wet conditions suggest that rat cementum is more mineralized than human cementum. This could indicate that in rats the substantial mineral content reinforces the collagen fiber bridges of the cement-dentinal junction (CDJ). It should be noted that although the collagen fibers forming bridges could be more mineralized in rats than in humans, the mode of attachment is similar ***(Sunita et al., 2009)***

The Cemento-enamel junction (CEJ) is the anatomical junction of enamel that coats a crown of the tooth and the cementum that coveres its root .The CEJ serves as an important point of reference in clinical dentistry. However, with increasing age , continuous passive eruption , which compensate s for wear at the incisal and occlusal surfaces along with the recession of the gingival tissue, results in a shift of the CEJ to the gingival sulcus. These changes expose the CEJ to the oral environment, thus making it vulnerable to pathological changes such as root caries and cervical erosion, resorbtion and abrasion .The mineralized tissues composing the CEJ presented four different interrelationships: cemetum over enamel, edge to edge relationship of cementum and enamel, gaps between cementum and enamel and enamel over cementum ***(Kapila et al., 2009).***

Dorsal surface of the tongue

The tongue is a sensitive, highly innervated, well-coordinated muscular organ responsible for moving food in the mouth .The tongue plays a vital role in food intake by vertebrates and so exhibits significant morphological differences in adaptation to the various diets and habitats (***Iwasaki and Asami, 2002).***

An indistinct groove, the sulcus terminalis divides the tongue into an anterior two thirds and a posterior one third. The anterior two thirds are covered with numerous papillae, which can be classified into four types: filiform, fungiform, foliate and circumvallate papillae. The posterior one third of the tongue is studded with small lymphatic nodules ***(Berkovitz et al., 2002).***

The dorsal surface of the tongue of the rodents can be divided into three parts: the anterior, the middle and the posterior parts. The anterior part contains the lingual apex and makes up almost half of the tongue. The middle part has a lingual prominence located at the inter-molar region close to the posterior half area of the tongue. The posterior part is a narrow region located at the lingual root ***(Kobayashi et al., 1989; Miyata et al., 1990)***.

Histologically, the filiform papillae consist of cone shaped structures, each with core of connective tissue covered by keratinized epithelium. With scanning electron microscopy, filiform papillae of human tongue have been seen to contain 5-12 hairs which are covered with a massive plaque of microorganisms. The surface of the body of the papilla is smooth and covered by epithelial cells that have a honey-comb pattern with prominent overlapping cell boundaries ***(Kullamikkon et al., 1987).***

The rat filiform papillae have been classified into three types , they are simple conical , giant conical ,and true filiform papillae (***Kaster and Cameron, 1978).***The simple conical papillae cover the anterior two thirds of the tongue .It forms a curved conical structure ending in a strong, cornified spine ***(Farbman, 1970).***The giant conical papillae form seven to ten rows, in the inter-molar eminence and separate the simple conical papillae from the true filiform papillae .The true filiform papillae occupy the posterior one third of the tongue .All of the filiform papillae possess a convex as well as a concave surface ***(kaster and Cameron, 1978)***.

There another classification of filiform papillae into three subtypes: non-branched conical filiform papillae(which were densely distributed on the surface of the anterior part of the tongue from the apex to the anterior border of the lingual prominence), spearhead-like filiform papillae distributed on the anterior margin of the lingual prominence, and small branched filiform papillae distributed on the posteriorly wide area of the lingual prominence (***Nonaka et al, 2008)***.

The second type of the lingual papillae is the isolated fungiform papillae found scattered between filiform papillae .In human they are elevated , mushroom -shaped which appear red because of their relatively thin non keratinized epithelium overlying a highly vascular connective tissue core .Taste buds are normally present in the epithelium of the superior surface ***(Nanci, 2003; Nonaka et al., 2008).***

The third type of lingual papillae is the circumvallate papillae .In human , several circumvallate papillae are usually seen anterior to the sulcus terminalis , and appear as large rounded papillae that do not project beyond the normal surface level of the tongue .Circumvallate papillae are surrounded by a deep circular trench into which the ducts of the minor salivary gland of Von Ebner open Taste buds are present in the epithelial wall of the trench ***(Berkovitz et al ., 2002).***

Each animal species has a varying number of vallate papillae; Rats, mice, and hamsters have one vallate papilla while rabbits, moles, suncuses, guinea pigs, horses and pigs have two vallate papillae (***Kobayashi et al., 1989; Miyata et al., 1990; Kobayashi, 1990; Kobayashi et al., 2005***). The rat circumvallate papilla is distinctive because it is circumscribed from the rear and lateral sides with a horseshoe –shaped, incompletely enclosed trench, so the left and right trenches do not merge in the anterior part ***(Aktas and Nergizy, 2009).***

In the rat tongue , Two foliate papillae are located on the posterolateral margin of the body of the tongue , composed of 4-5 ridges separated by deep parallel grooves or furrows, generally uniform in length and spacing (***Jackowiad and Godynicki, 2005).*** Foliate papillae that were oval-shaped with numerous parallel ridges and grooves were located on each side of the lateral margin of the lingual prominence (***Nonaka et al., 2008)***

Taste buds are chemoreceptive end organs for taste or gustation .In mammals , most taste buds are concentrated in the stratified squamous epithelium that covers the fungiform ,foliate and circumvallate papillae located on the tongue .Nonetheless , the cells of taste buds resemble typical epithelial cells in that they arise embryonically from the local epithelium. (***Stone et al ., 2002).***

<u>Mouthwashes:</u>

Many daily used drugs are characterized by causing physiological and pathological changes, all of which are directly relevant to dentistry ***(Brickley and Shepherd, 1990).*** The physiological and pathological aspects of some drug abuse on dental and oral tissues have not been examined in detail except for its effects on the oral mucosa ***(Harris et al., 2004).***

Mouthwashes are liquid preparations intended for being applied on teeth and mucosa of the oral cavity and pharynx in order to exert an antiseptic, astringent and sedative local action. They are widely used in dentistry and usually contain water with some active components, such as antiseptics, antibiotics, antifungal, astringents, and anti-inflammatory substances ***(Gaffar and Afflitto, 1992; Barbé et al., 1993)***.

The most common use of mouthwashes involves rinsing the mouth with about 20 ml of mouthwash two times each day after brushing .Typically the mouthwash is swished or gargled for about thirty seconds and the spat out (***Kozlovsky et al., 1996)***

<u>Types of mouthwashes:</u>

1. Antibacterial rinses (**Antiseptic mouthwashes)** have an antimicrobial effect in the entire mouth, including areas easily missed during tooth brushing and interdental cleaning. Studies show that antiseptics kill bacteria in saliva and on the soft tissues of the oral cavity, including the tongue and oral mucosa, which are reservoirs of pathogenic bacteria that transfer to and colonize on teeth ***(Pitts et al., 1981).*** For antibacterial rinses to be effective in the oral cavity they need to be bactericidal or bacteriostatic and most of all must have a degree of substantivity ***(Grossman, 1989).***

Janan et al., (2015) demonstrated that Chlorhexidine mouthwashes inhibited streptococcus mutans growth up to diluting concentration of 1:128. They advised Chlorhexidine as