

**A Clinicopathological Study of Focal Reactive Fibrous  
Overgrowths of Oral Mucosa and the Evaluation of  
Distribution of Collagen, Elastic and Reticular Fibers using  
Various Special Staining Methods**

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## **CERTIFICATE**

**This is to certify that the present study “ A CLINICOPATHOLOGICAL STUDY OF FOCAL REACTIVE FIBROUS OVERGROWTHS OF ORAL MUCOSA AND THE EVALUATION OF DISTRIBUTION OF COLLAGEN, ELASTIC AND RETICULAR FIBERS USING VARIUS SPECIAL STAINING METHODS” has been done by Dr. V Poornima, P.G. Student, Department of Oral Pathology and Microbiology, Tamilnadu Government Dental College & Hospital, Chennai, under my guidance and supervision. I am fully satisfied with her work.**

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# **CONTENTS**

## **TITLE**

- 1 INTRODUCTION**
- 2 AIMS & OBJECTIVES**
- 3 REVIEW OF LITERATURE**
- 4 MATERIALS AND METHODS**
- 5 RESULTS**
- 6 DISCUSSION**
- 7 SUMMARY AND CONCLUSION**
- 8. BIBLIOGRAPHY**

## INTRODUCTION

Human Oral Mucosa shows considerable variation in its structure, including the type of surface epithelium, form of the interface between the epithelium and the connective tissue, composition of the lamina propria, nature of the submucosa and the attachment of the mucosa to the underlying structures. The oral mucosa can be divided into three types:

1. The masticatory mucosa – Gingiva, Hard palate
2. The lining Mucosa – Buccal and Labial Mucosa, Floor of the mouth, ventral surface of tongue, alveolar mucosa, Lips and Soft palate
3. Specialised mucosa – Dorsum of tongue

Lamina propria is the connective tissue supporting the oral epithelium and is divided for descriptive purposes into papillary and reticular layer. These two layers differ essentially in the relative concentration and arrangement of various fibers. They are made up of cellular and intercellular components. The cellular components include fibroblasts, endothelial cells and inflammatory cells and the intercellular components are collagen, elastic and reticular fibers and ground substances.

Collagen fibers are the predominant component of the connective tissue and provide tone and form (stability and integrity). So far, 14 different types of collagen have been identified. Type I, III, IV & VII are the most common in oral mucosa.

Reticular fibers contain type III collagen, are finer and are connected to the coarser and stronger Type I collagenous fibers. They are localized to the basement membrane, blood vessels and surround smooth muscle cells.

Elastic system includes oxytalan, elaunin and elastic fibers, which have a fibrillar, mixed and amorphous structure respectively. They are thought to correspond to consecutive stages of normal elastogenesis. Elastic fibers consists of two distinct components an amorphous protein elastin and a microfibrillar structure termed as Elastic Fibre Microfibrillary Protein (EFMP). Elastic fibers are usually found in flexible lining mucosa where they restore tissue form after stretch.

Though these fibers vary in their composition, structure and arrangement, they are all produced by the same cell – “The Fibroblasts”.

Oral mucosa is subjected to both intrinsic and extrinsic stimuli and thereby manifest a spectrum of diseases ranging from developmental disturbances, reactive lesions, inflammatory lesions to neoplasms. They may be generalized or localized and may be due to localized or systemic cause.

Reactive fibrous hyperplasias of oral mucosa are lesions that commonly occur secondary to injury. They represent a chronic process in which an over exuberant repair follows injury. This group includes Fibrous epulis, Pyogenic granulomas, Calcifying Fibrous epulis, fibroepithelial polyp, Peripheral giant cell granuloma, Traumatic fibroma and Denture induced hyperplasia. These are more common in the gingiva followed by buccal mucosa, lip, tongue and palate. Most of these lesions share common clinical features , but have diverse or varied histopathological features. The reason for these over exuberant repair is unknown. Any stimuli that alter the fibroblastic activity will result in the alteration of the fibre type produced.

The distribution of different connective tissue fibers in focal reactive fibrous hyperplasias of oral mucosa is not clearly understood. Therefore, a detailed study is taken up to analyse the distribution of different connective tissue fibers in focal reactive fibrous hyperplasias of oral mucosa.

## **REVIEW OF LITERATURE**

### **CONNECTIVE TISSUE FIBRES:**

Connective Tissue with the exception of adipose connective tissue is characterized by a relative paucity of cells and a predominance of fibres and amorphous jellies. Unlike epithelium, the connective tissue cells are not polarized and have no close spatial association with each other. The jelly components of the intercellular substances are frequently dissolved in histological sections and it is important to remember that the spaces in such sections were filled in life with the amorphous elements and tissue fluid.

**LEESON C.R., LEESON T.S., PAPPARO A.A. (1985)** <sup>[39]</sup> proposed in their textbook of histology that the fibrous intercellular substances which includes collagen, reticular and elastic fibres provide strength and support for the tissues. He proposed that all are complex proteins formed by the long chain of amino acids, i.e., polypeptides and are comparatively insoluble in neutral solvents which explain their ability to exist as formed fibres in the fluid environment of the body.

**CORMACK D.H. (1987)** <sup>[22]</sup> described that a microscopic preparation that shows the fibres in loose connective tissue to considerable advantage can easily be made from a small tissue sample that is removed from superficial fascia under the skin, stretched out flat to make a preparation called a spread and then fixed and suitably stained to reveal its various components.

**KESSEL R.G (1998)** <sup>[35]</sup>. in their text book of Basic Medical Histology proposed that the connective tissue fibres are of three types:

- Collagen Fibres
- Elastic Fibres
- Reticular Fibres

Connective tissue is classified into embryonic and adult connective tissue. Collagen, elastic and reticular fibres are classified under adult connective tissue and they are further classified into various groups:

**A. Loose (Alveolar) ordinary Connective Tissue:**

Loosely organized, widely distributed, consists of mixture of cells and intercellular substances. Found in submucosa and lamina propria layers of organs. All fibre types present

**B. Reticular connective tissue**

**C. Dense, ordinary connective tissue.**

**Regularly arranged when the fibres are aligned and closely packed**

- a. White fibrous connective tissue (collagen fibres) contains fibroblasts and collagen
- b. Yellow elastic connective tissue (elastic fibres) mainly elastic fibres and fibroblasts.

**Irregularly arranged (when fibres are haphazard in orientation)**

- a. Dense white fibrous connective tissue (collagen fibres)
- b. Dense, yellow elastic fibres (elastic fibres). Present in blood vessels.

**CONNECTIVE TISSUE OF ORAL MUCOSA:**

**WENTZ F.M, MAIER A.W. (1952)** <sup>[75]</sup> investigated that the age changes and sex differences in the clinically normal gingival and reported that epithelial

cells do not show any significant changes with age though sex changes were observed in the epithelium and connective tissue of 15 – 29 years age group which showed a medium suprapapillary width and long ridges with fairly textured fibres in papillary layer of connective tissue in females and narrow suprapapillary width and long ridges and coarsely textured fibres in males. The connective tissue elements showed down in cellularity with increased fibrous Intra cellular substance and coarsely textured fibres with advancing age.

**COHEN B., KRAMER I.R.H. (1976)** <sup>[20]</sup>, in their text book – Scientific Foundations of Dentistry proposed that connective tissue comprises the bulk of the gingival tissue by volume. It occupies approximately 70%. Its most important and voluminous constituents are collagen fibrils, which occupies 56% of the total connective tissue fraction.

Masticatory mucosa covers hard palate and most regions of the gingiva. Lining mucosa constitutes the oral surface of the cheek and lips, buccal and labial sulci, floor of the mouth, ventral surface of tongue and soft palate. Specialized mucosa lines the dorsal surface of the tongue. Thus masticatory mucosa covers the lining of the immobile regions and lining and specialized mucosa cover mobile regions.

The fibres of the lamina propria carry a large share of the protective functions of the mucosa. Reticular, collagenous and elastic fibres vary in amount in different parts of the mucosa as fits regional needs. Fibroblasts produce required quantities of these fibres, as well as the glycoproteins of the ground substance of lamina propria, they are also the source of regionally different determinants for the overlying epithelium and perhaps of chalone regulating not only their own mitotic rates but influencing that of epithelium as well.

**TENCATE A.R. (1998)** <sup>[72]</sup> summarized the structural variations in the oral mucosa and the nature of the submucosa and how the mucosa is attached to the

underlying structures in various regions. The organisation of component tissues showed similar patterns in many regions. He found that the lamina propria of masticatory mucosa is thick containing dense network of collagen fibres in the form of large, closely packed bundles. They followed a direct course between anchoring points so that there is relatively little slack and the tissue does not yield on impact enabling the mucosa to resist heavy loading.

The lamina propria of lining mucosa is generally thicker than masticatory mucosa and contain fewer collagen fibres, which follow a more irregular course between anchoring points. Thus the mucosa can be stretched to a certain extent before these fibres become taut and limit further distension.

Where lining mucosa covers muscles it contains a mixture of collagen and elastic fibres. They retract the mucosa towards the muscle as they slack during masticatory movements. So that, it prevents it from bulging between the teeth and being bitten.

### **COLLAGEN FIBRES - STRUCTURE, SYNTHESIS & COMPOSITION**

**CULLING C.F.A. (1963)** <sup>[23]</sup> published that there are types of fibres in the connective tissue which includes white / collagen, yellow / elastic and reticulin fibres. He proposed that collagen fibres may appear as bundles of wavy / straight fibres, which do not branch and run in every direction. On boiling it yields gelatin, swells in acetic acid and digested by pepsin.

They also proposed that a good routine stain for histological diagnosis must stain selectively not only cell nuclei and cytoplasm but also connective tissue. Haematoxylin and Eosin, if properly differentiated will distinguish nuclei, which appear blue and cytoplasm and connective tissue fibres that appears as shades of pink. But, sometimes, we prefer a preparation in which connective tissue fibres are stained various colours instead of differing shades of one colour and this led to the development of Various methods for demonstrating connective tissue components

such as Van Gieson either as a counterstain with iron Haematoxylin or following on the elastic fibre stains.

Other methods described are Heidenhain's azan stain, Lilies allochrome method, Masson's and Mallory's Trichrome, Lendrum's acid – picro Mallory, Lison's alcian blue chlorantine fast and Mallory's Phospho Tungstic Acid Haematoxylin.

**BARKER D.S., LUCAS R.B., (1967)** <sup>[5]</sup> studied the localised fibrous overgrowths of oral mucosa occurring palate, lip, tongue, cheek have observed that these are two fibre patterns occurring in these lesion. The circular pattern occurring in mobile mucosa and are with low degree of irritation. The radiating pattern occurring in the fixed mucosa and are with greater degree of irritation.

**KFRR D (1968)** <sup>[37]</sup> studied the incidence and distribution of precollagenous reticulin fibres, collagenous fibres, and oxytalan fibres in a sample of oral fibromas and reported that the collagenous stroma showed much variation in fibre size and orientation.

**GARANT P.R. (1976)** <sup>[27]</sup> studied collagen resorption by fibroblasts and reported that fibroblasts must be viewed as a dynamic entity capable of migration, phagocytosis, degradation of polymerized collagen rather than a stationary collagen secreting cell and as such a key element in the maintenance as well as formation of periodontal ligament. They mentioned that Tencate was the first to publish and clearly demonstrate the extensive distribution of intracellular collagen in oral connective tissue fibroblasts and proposed a hypothesis that intracellular collagen fibrils represent phagocytosed collagen destined to undergo enzymatic digestion within secondary lysosomes.

**BARTOLD P.M., WIEBKIN O.W., THONARD J.C. (1982)** <sup>[6]</sup> biochemically analysed molecular size distribution of proteoglycans in the epithelium and connective tissue of human gingiva and noted that the epithelial

proteoglycans are able to self aggregate to form large macro molecules and are larger than the connective tissue proteoglycans. They proposed that the connective tissues matrix would be more susceptible to bacterial and endogenous enzyme degradation and to immunological inflammatory invasion than the more concentrated and complex network of the epithelial extracellular matrix, though the mechanism of penetration into the connective tissue to bring about tissue destruction is not clearly understood.

**GARNICK J.J., WALTON R. E., (1984)** <sup>[29]</sup> studied the fibre system of facial gingiva in animal model and reported that they consists of two principal components (a) well developed horizontal fibres (b) cemental fibres and proposed that it cannot be stated in absolute terms that the same morphology and function will be present in humans.

**CHAVRIER C., COUBLE M.L., MAGLOIRE. H AND GRINAND J.A. (1984)** <sup>[18]</sup> discussed the morphological pattern of connective tissue organisation of healthy human gingiva and the nature of its collagenous components. There are morphologically two types of organisation with different collagenous component. Pattern I appears to be predominant type of organisation of gingival connective tissue and composed of large bundles of long, thick, striated collagen fibres. (Type I fibres) and are associated with the stability. Pattern II appears to be present underlying gingival basement membrane or surrounding basement membrane of blood vessels and composed of short thin, striated collagen fibres and non-striated fibrillar material (type III) and is associated with remodelling ability under physiological and pathological conditions. Type IV collagen is exclusively limited to Lamina Densa of the basement membrane.

**JOHNSON B.D., GUINDY M.EL, AMMONS W.F., NARAYANAN S.. AND PAGE R.C. (1986)** <sup>[33]</sup> compared fibroblasts from an unidentified syndrome with gingival hyperplasia as the predominant feature with fibroblasts from

phenytoin induced gingival hyperplasia and age and sex matched control strain and reported that it is clear that the three cell types are phenotypically different and these differences persist through generations and transfers in culture. The abnormalities in synthesis may or may not be directly related to the clinical condition. They also reported that drug-induced and idiopathic gingival hyperplasia though appear clinically similar there exists difference in synthetic activities of cells from these conditions.

**LEESON C.R., LEESON T.S., PAPPARO A.A.(1985)** <sup>[39]</sup> stated that in fresh state collagenous fibres are soft and flexible, relatively inelastic of high tensile strength. They are transparent and homogenous. They are birefringent under polarized light, indicating longitudinal orientation of subunits or fibrils.

They proposed that in addition to the microfibril with a periodicity of 640 amstrong (64 nm) characteristic of native collagen, it also exists in long spacing form with a periodicity of 2400 amstrong (240 nm), in which there are two varieties. Fibrous long spacing form (FLS) found in the trabecular meshwork of the eye and aging cartilage and Segment Longitudinal spacing type (SLS). FLS collagen is formed by rows of tropocollagen units lying end to end in parallel – antiparallel array without overlap and SLS collagen is formed by lateral and lengthwise aggregation of similar units.

**CORMACK D.H., (1987)** <sup>[22]</sup> proposed in their textbook – Ham's Histology that the collagen fibres are characteristically thick and unbranched with a diameter of 2 – 10  $\mu$  and in spread preparations they are wavy. Several fibres may be collected together to form a bundle of greater size, within a bundle fibres are held together by a small amount of amorphous cement substance (mucoprotein). The fibres are made up of fibrils of approximately 50 nm in width which are in turn composed of smaller microfibrils. The microfibrils exhibit a banding pattern with an axial periodicity of 640 Å (64nm).

**CHO M II, GARANT P.R., AND LEE Y.L. (1988)** <sup>[19]</sup> studied fibronectin rich contact sites on fibroblasts of normal periodontal ligament and inflamed gingiva by immunocytochemistry and routine EM. They found that fibroblasts to matrix attachment sites in healthy periodontal ligament were rectangular patches of amorphous material juxtaposed to the external surface of plasma membrane and in inflamed gingiva the amorphous material and fibronectin associated to the cell surface. They reported that the functional significance of the difference between fibroblast to matrix contacts in healthy and inflamed connective tissue remain to be established. They honour reported changes in cell motility.

**NARAYANAN A.S., MEYERS D.F. AND PAGE R.C. (1988)** <sup>[50]</sup> studied how collagen production is regulated in fibroblasts obtained from normal and phenytoin induced hyperplastic human gingiva and reported that over production results from an increased steady state levels of collagen m-RNA and not decreased collagen degradation. They also reported that lysosomal inhibitors do not enhance collagen production in both normal and phenytoin cells.

**Luder HU , ZIMMERLI I., SCHROEDER H.E (1988)** <sup>[41]</sup> quantitatively estimated the diameter of over 25000 periodontal ligament collagen fibrils and demonstrated that the diameter of average collagen fibril i.e. 54 – 59 nm is rather constant throughout human life span especially true for large fibrils (57 – 64 nm) while a numerically limited population of small fibrils (i.e. 40 – 48 nm) increase in diameter with age.

They also stated that type III collagen has been suggested to regulate the elasticity and flexibility of a connective tissue and decrease in the proportion of small fibrils points to an age dependent increase in stiffness of the periodontal ligament. They quoted that depending on the type of tissue, age and species the collagen fibril diameter varies between 15 and 300 nm and may also be related to the type of amount of Glycose Amino Glycans in the surrounding matrix and also

to the functional status of tissue involved. A comparatively small fibril diameter (about 50 nm) was found in tissues functioning under compressional stress while larger collagen fibril diameter may be typical for tissues existing under a variable tensional load. Their study showed that the increase in the average diameter of human skin collagen fibrils from fetal to adult age is associated with a decrease in ratio of type III / type I collagen.

**MC. CULLOCH CAG, BORDIN S (1991)** <sup>[45]</sup> analysed and reviewed the role of fibroblast subpopulations in periodontal physiology and pathology indicated that there are site specific phenotypic variation of fibroblasts in health and disease. But it has not yet been established whether observed variations in collagen activities progression of the disease or to other factors such as modulation of fibroblast synthetic activities by environmental ligands. They suggested that pathologic alterations of oral connective tissue may result from a clonal imbalance of resident fibroblast subtypes rather than the presence of abnormal cells. It was mentioned that the specificity of fibroblast phenotype may influence not only the metabolism of the connective tissues but also of adjacent epithelia. The regional specificity of epithelia may depend at least in part on the phenotype of underlying fibroblasts. The range of behaviour expressed by fibroblasts substrains under changing environmental stimuli is intrinsic in the cell. Under optimal conditions the genotype may be chiefly controlling proportions of the various fibroblasts subtypes in oral tissues, but environmental factors may be more important regulators under sub optimal conditions.

**RABANUS J P, GELDERBLOM H R, SCHUPPAN D, BECKER J (1991)** <sup>[59]</sup> investigated the ultrastructural localization of collagen type V & VI in normal human alveolar mucosa by immunoelectron microscopy. They reported that the collagen type V were located in larger spaces between cross striated major collagen fibrils as uniform non – striated microfibrils of 12 – 20 nm in width and

also occasionally single fibrils fanned out from the ends of major collagen fibrils which may indicate a role as core fibril.

Collagen type VI was detected in a loose reticular network of unbanded microfilaments in the vicinity but not as an intrinsic component of the sub epithelial basement membrane. They appear to form bridges between neighbouring cross striated major collagen fibrils, suggesting an inter connecting role for this collagen type. They morphologically appeared as knob like projections every 100 – 110 nm.

**BECKER J, SCHUPPAN D., MULLER S (1993)** <sup>[49]</sup> in their study on immunohistochemical distribution of collagen type 1, 3, 4 & 6, undulin and tenascin in oral fibrous hyperplasias have found that oral fibrous hyperplasias that are probably caused by inflammation or chronic irritation show the differentiated and ordered pattern of EMP characteristic of oral mucosa.

**YAKOVLEV E., KALICHMAN I, PISANTI S, SHOSHAN S & BARRAK V (1996)** <sup>[77]</sup> examined the age dependent relationships between levels of inflammatory cytokines and collagen in human gingiva inflammation and reported that level of type 1 collagen showed no difference between inflamed and non – inflamed gingiva in any age groups, whereas the level of type 3 collagen was lower in inflamed than in non – inflamed gingiva in both children and older age groups.

**MIGHELL A J, ROBINSON P A., HUME W J., (1996)** <sup>[47]</sup> observed variation in the immunoreactivity of tenascin – C in areas with similar light microscopic appearances and they proposed that stained fascicles may reflect areas of active tissue organisation, whereas unstained fascicles possibly represent quiescent areas of tissue.

**BANCROFT J D., STEVENS A., (1996)** <sup>[4]</sup> proposed that on electronmicroscopy type 1 collagen are tightly packed thick fibrils with little

interfibrillar substance. The prominence of cross banding is thought to be due to lack of interference from interfibrillar ground substance. The presence of partially processed type 3 pre collagen pN collagen 3 helps to regulate the diameter of fibrils formed by collagen type 1, by forming co – polymers with the fibrils. pN collagen 3 inhibits the rate at which collagen type 1 is assembled into fibrils and also decreases the amount of type 1, which is incorporated into the fibrils.

They described that type 3 collagen are found only in tissues that contain type 1 collagen. Type 3 collagen was misleadingly called as fetal collagen because they are also present in adults. They constitute 60% of fetal collagen, but only 20% in adults. They allow some mutility and easy diffusion and exchange of metabolites.

Type 4 collagen, reveals a random organisation of very fine fibrils forming a felt like structure in all basement membrane. They are closely associated with significant amount of carbohydrate complexes, which explains the strong reaction of basement membrane by PAS method.

**KESSEL R G., (1998)** <sup>[35]</sup> described that collagen is synthesisd on membrane ribosomes in fibroblasts and appear in rER cisternae as pro alpha chains where a number of post – translational changes takes place which includes:

- Cleavage of the signal peptide
- Hydroxylation of glycine, proline and lysine residues
- Glycosylation of some portion of hydroxylysine residues
- Formation of triple helix by 3 pro alpha chains
- Formation of S – S bond that shape the molecule and provide stability.

Resulting in the formation of procollagen which are packaged into secretory vesicles in golgi region and discharged by exocytosis to the extra cellular space. There are extra amino acids called propeptides when  $\alpha$  - chains are first synthesized and these are thought to prevent the formation of collagen fibers in the

cells. After release to the exterior these are removed by proteolytic enzymes called procollagen peptidase, thus procollagen becomes tropocollagen. Tropocollagen forms fibrils. Glycine, proline and hydroxylysine are essential for the formation of triple helix and some sugar groups may be covalently attached to the hydroxylysine residues. Hydroxyl groups in hydroxyproline are essential for the stability of triple helix.

Type 4 collagen differs from other collagens in that the amino acids sequence of  $\alpha$  - chains is interrupted in several regions to disrupt the triple stranded helix and propeptides are not removed from the procollagen molecule after secretion.

**TENCATE A R., (1998)** <sup>[72]</sup> stated collagen comprises about 30% of all body proteins. 14 different types of collagen have been identified that are genetically, chemically and immunologically distinct. Variations in collagen fibers are brought about by

- Differences in the length of the helix
- Differences in the assembly of the basic polypeptide chains
- Various interruptions in the helix
- Differences in the termination of the helical domain

### **ELASTIC FIBERS**

**CULLING C F A, (1963)** <sup>[23]</sup> proposed that elastic fibers occur singly, branch and are highly refractile. They are unaffected by boiling, acetic acid or pepsin maybe digested with trypsin. Gomori's aldehyde fuschin is the most reliable method for the demonstration of elastic fibers, but Verhoeff's is probably the easiest to prepare and most permanent, Weigert's resorcin – fuschin (or Hart's modification ) gives excellent results provided a good batch of basic fuschin is available.

**KERR D (1968)** <sup>[53]</sup> studied the incidents and the distribution of precollagens reticulin fibers, collagenous fibers, elastic fibers and oxytalan fibers

in a sample of oral fibromas and reported that the presence of elastic fibers indicated that they may arise on an inflammatory or reactive basis and elastic and oxytalan fibers showed similar incidence and distribution.

**COHEN B., KRAMER I R H., (1976)** <sup>[20]</sup> proposed that elastic fibers consist of an electronlucent amorphous core of light microscopic dimension and numerous fine tubular filaments forming acute angles with the core. Elastic fibers branching in close contacts with the ends of collagen fibers have been demonstrated in oral mucosa. He proposed that oxytalan fibers maybe immature elastic fibers rather than a distinct fiber type.

**PORTER K., DOONER J J., LOPEZ A., (1977)** <sup>[57]</sup> have reported in their second study after criticism of their first study published in 1976 that elastic fibers are present in both the papillary and reticular connective tissue of attached human gingival and also in the adjacent oral mucosa.

They have also confirmed that elastic fibers stained with acid – orcein giemsa stain both with and without treatment with borate buffer. But these fibers failed to stain after treatment with elastase in borate buffer.

**WRIGHT B A., (1979)** <sup>[76]</sup> did a histochemical study of peripheral odontogenic fibroma and found that oxytalan fibers is present in all of these cases and so they are of periodontal ligament origin. The oxytalan fibers have to be differentiated from pre elastic fibers only by electronmicroscopy and there is no evidence yet to suggest that elastic fibers are to be found embedded in the bone.

**LEESON C R., LEESON T S., PAPARO A A., (1985)** <sup>[39]</sup> published that in contrast to collagen fibers elastic fibers appear homogenous and non fibrillar by light microscopy and in the fresh state adult elastic tissue in bulk has a yellowish colour. If fresh tissue is treated with dilute acidic solutions, collagen fibers swell and become transparent but elastic fibers become visible as highly refractile, homogenous shiny threads.

**CORMACK D H., (1987)** <sup>[22]</sup> proposed though the amino acid composition of elastin is similar to that of collagen, the proportion of charged amino acid is considerably lower. Elastin is rich in proline and glycine but it contains little hydroxyproline and lacks hydroxylysine. In addition it contains Desmosine and Isodesmosine that crosslinks tropoelastin molecules.

Lysyl oxidase present in the extracellular space links the lysine group of 4 tropoelastin molecules together during the formation of elastin. Elastin is believed to be made up of cross linked flexible peptide chains that by entering into labile interactions can cause transient change in the internal configuration of the protein. Electronmicroscopic studies by Gotte L, (1980) of purified elastin indicate that it contains interwoven bundles of paired filaments running roughly parallel to the long axis of the fiber.

**BANCROFT J D., STEVENS A., (1996)** <sup>[4]</sup> described that the elastin system fibers oxytalan, elaunin and elastic fibers have a fibrillar structure (oxytalan fibers), an amorphous (elastic fibers ) or a mixed structure (elaunin structure). Elastic fibers consists of 2 distinct components

- An amorphous component consistent biochemically with protein elastin
- Microfibrillar structure with a periodicity of 14 – 13 nm and termed as Elastic Fiber Microfibrillar Protein (EFMP) or Elastin Associated Microfibrills

EFMP – believed to provide tensile strength and flexibility to numerous tissues and act as a scaffold for elastin deposition. The 2 components seems to alter with the age of the fiber in young fibers, the dominant fraction is the microfibrillar protein, in older fiber, the amorphous protein accounts for more than 90% of the fiber content. The basic molecular unit of elastin is a linear polypeptide of molecular weight of approximately 72 KD and referred to as soluble elastin or tropoelastin. These polypeptides are transported out of the fibroblasts or smooth

muscle cells and the cross-linking occurs in the extra cellular spaces, by means of crosslinking compounds Desmosine and Iodesmosine.

The EFMP has an aminoacid content quite different from that of elastin protein and has an increased content of cystine which has numerous disulphide linkages. They also contain no. of carbohydrate complexes termed as “Structural Glycoproteins” which also has a significant role in staining. Young fibers with increased content of EFMP show positive PAS reaction. With increasing age the fibers split, fragment and there is alteration in the ratio of EFMP: Elastin and increased levels of glutamic acid, aspartic acid and calcium. These changes are readily visible in the skin, which also becomes loose and wrinkled.

They also stated that elastin and pre – elastin fibers are highly crosslinked by disulphide bridges which following oxidative treatment eg. Potassium permanganate in weigert type methods, iodine in Verhoeff’s hematoxyline be in part converted to anionic sulphonic acid derivatives. These are strongly basophilic and capable of relatively selective reactions with basic dye components.

**GOLDSTEIN (1962)** stated that elastic tissue staining by orcein , resorcin – fuschin was reduced or inhibited by the presence of urea, a strong hydrogen bonding agent. He considered that if hydrogen bonding was responsible for elastic staining, the stain molecule must be hydrogen donor and the tissue the hydrogen acceptor.

**BHASKAR S N (1997)** <sup>[54]</sup> stated that elastic fibers which are elaborated by fibroblasts and also possibly by smooth muscle cells in the walls of blood vessels. Unlike collagen and reticular fibers, they are not considered to be important constituents of the fully repaired tissues.

They are stained by aldehyde fuschin, resorcin – fuschin and specifically by orcein in histological preparations. A fluorescent staining method using

tetraphenylporphine sulphonate in combination with silver or gold has been developed by Albert and Fleicher for electronmicroscopic visualization.

The lining mucosa in lips, cheeks where they cover muscle is fixed to the epimysium or fascia and are highly elastic. The lamina propria has dense connective tissue and short irregular papillae. The submucosa consists of strands of densely grouped collagen fibers, which limit the mobility of the mucus membrane.

## **RETICULAR FIBERS**

**CULLING C F A, (1963)** <sup>[23]</sup> stated that reticular fibers in contrast to collagen fibers are Isotropic (not birefringent), gram negative and coloured black by silver techniques. They are thought by some to be immature collagen fibers, others thought it to be physically and chemically different.

Reticular fibers are best demonstrated by silver impregnation. Gomori silver reticulin method is recommended for its simplicity and reliability. Sections to be treated with silver impregnation should always be fixed to the slide with starch paste or glycerin albumin.

**KERR D., (1968)** <sup>[37]</sup> reported that active growth or proliferation of the collagenous portion of the fibrous connective tissue stroma in the oral fibromas was not occurring as indicated by the absence of precollagenous reticular fibers.

**COHEN B., KRAMER I R H., (1976)** <sup>[20]</sup> proposed that reticular and collagenous fibers are both thought to be secreted as tropocollagen molecules undergoing, when outside the cells, lateral and longitudinal aggregation to reticular and collagen fibers respectively as well as association with glycoproteins.

**CORMACK B H., (1987)** <sup>[22]</sup> described that reticular fibers are fine and delicate, branch extensively to form a delicate supporting network (L. Rete – Net). By impregnation with silver, reticular fibers stain black and collagen yellow to brown. He explained that the basis of silver impregnation technique is that the tissue is treated first with a reducible silver salt and then with a reducing agent.

The reducing agent acts like a photographic developer, reducing the silver salt to metallic silver, which appears black. In loose connective tissue they are believed to be produced by the fibroblasts, but in haematopoietic tissues, they appear to be produced by reticular fibers.

**LEESON C R., LEESON T S., PAPARO A A., (1985)** <sup>[39]</sup> stated that reticular fibers occur as fine networks around blood vessels, muscle fibers, nerve fibers, fat cells, in the fine partitions of lung and particularly in the boundaries between connective tissue and other types of tissue (eg. Basement membrane). They are not birefringent and on electron microscopy show the periodicity of 64 nm characteristics of collagen fibers. But they stain more densely periodic – acid – schiff PAS technique than collagen fibers due to the high content of hexoses in reticular fibers – 6% or more as opposed to 1% in collagen fibers, which is insufficient for them to be stained by the procedures.

**BANCROFT J D., STEVENS A., (1996)** <sup>[4]</sup> stated that reticular fibers are connected to coarser collagen fibers. They are weakly birefringent because of their lack of physical size and the masking effect of the interfibrillar substance. The demonstration of these fibers by Silver reticular method in paraffin sections and PAS in frozen sections depends on the reactive groups in the carbohydrate matrix and not upon the fibrillar elements of the fiber.

**BHASKAR S N (1997)** <sup>[54]</sup> stated that the newly elaborated collagen fibers formed during development or in wound healing are equivalent to reticular fibers in their electronmicroscopic structure. Both of these fibers stain positively for glycoproteins with silver stains and a PAS method.

They also stated reticular fiber distribution in the lamina propria of the masticatory mucosa and lining mucosa differ in that the buccal mucosa contains more reticular fibers and its papillae are high and more closely packed.

## **FOCAL REACTIVE OVERGROWTHS OF ORAL MUCOSA FIBROUS EPULIS**

**BERNICK S (1948)** <sup>[7]</sup> reported that chronic inflammatory growth constituted 26% of so-called epulides and are common in females (70%) in the age range of 7 – 75 years and more common in maxilla. These inflammatory lesions were usually situated about or between the teeth, small but maybe large enough to cover two or more teeth. On histopathology there is increase in all the elements comprising the gingival tissue, increase in fibroblast, epithelial papillae and collagenous fibers in the submucosa and infiltration by small lymphocytes and plasma cells.

**COOKE B E D., (1952)** <sup>[21]</sup> studied the histogenesis and the natural history of fibrous epulis and fibroepithelial polyps and reported that the difference between fibroepithelial polyp in the gingiva and fibrous epulis is that the former is purely a reparative process while the latter is a reparative process in association with inflammatory changes.

**WILLIS R A.,** stated that the cellular proliferation of the reparative tissues unlike that of tumors, is not progressive and continuous but is limited by the extent of the breach to be filled or the duration of the tissue damage evoking it.

**RAMFJORD S., ARBOR A, MICH (1952)** <sup>[60]</sup> designated the term fibroid epulis and describes it as a localized non specific non neoplastic fibrous gingival hyperplasia and found that the stimulation to growth is most marked around areas of inflammatory infiltrations commonly seen in crevicular regions and base of the surgically removed lesions.

**SALMAN I, LANGEL I (1953)** <sup>[64]</sup> discussed the benign soft tissue tumors of oral cavity and reported that these are of either connective tissue or epithelial tissue in origin and majority of these comprises connective tissue origin. Many of

the soft tissue tumors resemble each other and true diagnosis can be made only from microscopic examination of the tissue after removal.

**BHASKAR S. N., LEVIN M., (1973)** <sup>[9]</sup> studied the histopathology of 1269 biopsies of human gingival and reported that within the limitations of their study, the gingival lesions vary from plasmacell granuloma, edematous gingivitis, fibrous gingivitis, pyogenic granuloma, peripheral fibroma with and without calcification, giant cell reparative granuloma, desquamative gingivitis, gingival cyst, sarcoidosis and suggested that whenever the gingival lesions are encountered biopsy should be taken especially if they do not respond to treatment

**KFIR Y, BUCHNER A, HANSEN L. (1980)** <sup>[36]</sup> reported a clinicopathological study of 741 cases of reactive lesions of gingival and indicated that there are some differences in age and sex distribution as well as in location and size of the lesions. These lesions include pyogenic granuloma, peripheral giant cell granuloma, peripheral fibroma with calcification and fibrous hyperplasia. They reported that pyogenic granuloma and peripheral fibroma with calcification occur in younger patients more often than fibrous hyperplasia and thus may represent a stage in the development of fibrous hyperplasia.

**STABLEIN M, SILVERGLADE L (1985)** <sup>[69]</sup> analysed 834 biopsy specimens from gingival and alveolar mucosa to see if differences in structure and function are reflected in categories and frequencies of diagnosis. They reported that inflammatory or reactive hyperplasia accounted for about 85% of the lesions and neoplasia most of the remainder. Pyogenic granuloma was the most common lesion in gingival (23.6%) and fibrous hyperplasia in the alveolar mucosa (23.2%). They concluded that the lesions in these two regions were similar but differed greatly in their frequency and age and sex distribution.

**MIGHELL AJ., ROBINSON PA., HUME WJ.(1996)** <sup>[47]</sup> observed marked variation in the spatial distribution and intensity of tenascin – C

immunoreactivity within individual specimen of pyogenic granuloma, fibrous epulis, calcifying fibrous epulis and peripheral giant cell granuloma. This reflects areas of active tissue morphogenesis and cell migration and suggests a difference in connective tissue composition of fibrous lesions such as fibrous epulides compared to fibroepithelial polyps, denture induced hyperplasia and giant cell fibromas.

**PHILIP S, EVERSOLE L R., WYSOCKI G P (2004)** <sup>[56]</sup> described that benign soft tissue growths found within the oral cavity are the result of reactive hyperplasia and are composed primarily of one or more of the following connective tissue components: mature collagen fibers, focal bone formation, endothelial cells and multinucleated giant cells. When this focal reactive connective tissue proliferation is confined to the gingival and its exact histological nature is unknown it is clinically designated as an “epulis”. The most common lesions referred to as epulides are peripheral fibroma, peripheral ossifying fibroma, pyogenic granuloma, and peripheral giant cell granuloma.

### **PYOGENIC GRANULOMA**

**BERNICK S (1948)** <sup>[7]</sup> reported that pyogenic granuloma, comprised 5.5% of the so called epulides in their study and the main causative factors were local traumatism, injury and infection in addition to systemic or constitutional condition tending towards the proliferation of gingival tissue.

**KERR D., ARBOR A (1951)** <sup>[37]</sup> reviewed the literature and correlated in their review of 289 cases and concluded that granuloma pyogenicum and their similarity with keloid and so called pregnancy tumour indicates that systemic factor which stimulates vascular proliferation plays the part in the etiology of this lesion. This lesion is common in mucous membranes as skin and therefore is considered as a blastomatoid lesion, which may involve any surface of the body.

**BOYLE P, (1952)** <sup>[12]</sup> reported the differential diagnosis of soft tissue lesions of the mouth with a discussion of biopsy procedure. In their study on a total of 1510 surgical specimens inflammatory and reparative processes including granuloma pyogenicum and denture induced hyperplasia constituted 402 cases. One case clinically diagnosed as pyogenic granuloma proved to be small but definite epithelial neoplasm on microscopic examination.

**SHARAWY A. M, LOBENE R (1968)** <sup>[67]</sup> reported a case of pyogenic granuloma occurring in a rats gingiva and demonstrated the presence of gram +’ve and gram – ‘ve cocci only on the surface and their absence at the base of the lesion and so they ruled out that bacteria were not a factor in the etiology of this lesion and they were contaminants from the oral mucosa.

**ANGELOPOULOS A. P. (1971)** <sup>[2]</sup> analysed 46 new and 789 previously reported instances pyogenic granuloma of oral cavity statistically. He proposed the term hemangiomatous granuloma, which expresses the characteristic of histopathological picture (hemangioma like) and inflammatory nature (granuloma) of the lesion.

**HATZIOTIS J (1972)** <sup>[31]</sup> analysed the incidence of pregnancy tumor and their possible relation to embryo’s sex and reported that a statistically significant result indicating that pregnancy tumour was more often associated with male infants. No explanation could be given for this finding.

**BHASKAR S. N., LEVIN M., (1973)** <sup>[9]</sup> reported 170 cases of pyogenic granuloma in their series of 1269 gingival biopsies and reported the rare observance of giant cells and foci of calcification in 3 and 5 cases and no malignant or local aggressive behaviour in any case.

**VILMANN A., VILMANN P., VILMANN H. (1986)** <sup>[74]</sup> discussed the etiopathogenesis of pyogenic granuloma and proposed that pyogenic granuloma maybe a localized tissue response to non specific irritant such as trauma,

inflammation, microtrauma or local irritation. This non-specific irritant some kind of tissue organizer, which might be a C type virus.

**NEVILLE B W., DAMM D D, ALLEN C. M., BOUQUOT J E. (1995)** <sup>[51]</sup> reported that pyogenic granuloma inspite of its name is not a true granuloma and observed in gingival, buccal mucosa, lips and tongue. A history of trauma is usually not uncommon especially for extra gingival lesions.

Pregnancy tumour or granuloma gravidarum begin to develop during the first trimester and their incidence increased through the seventh month of pregnancy and may be related to the increased levels of estrogen and progesterone as pregnancy progresses, after which they are either resolve spontaneously or undergo fibrous maturation or fibroma. They describe that pyogenic granuloma microscopically shows high vascular proliferation that resembles granulation tissue with numerous large and small endothelium lined channels that are engorged with RBCs and organised in lobular aggregates. Some pathologists require this lobular arrangement for the diagnosis.

**LAWOYIN J. O., AROTIBA J. T., DOSUMU O.O., (1997)** <sup>[38]</sup> compared pyogenic granuloma of oral cavity in Africans with that of others and they found more significant difference except for the occurrence of large lesions because of late presentation due to decreased awareness.

**CAWSON R. A., BINNIE W.H., SPEIGHT PAUL, BARREH A. W., WRIGHT J. M. (1998)** <sup>[16]</sup> proposed that microscopically pyogenic granulomas contain numerous large thin walled blood vessels that they were alternatively called granuloma telangiectaticum.

**REGEZI J A., SCUIBBA J J (1999)** <sup>[63]</sup> published in their textbook of clinical – pathological correlations that pyogenic granuloma is the misnomer in that it is not pus producing as pyogenic implies and is a tumour of granulation

tissues as granuloma implies and has to be differentiated from peripheral giant cell granuloma and peripheral fibroma, for which biopsy is definitive.

### **CALCIFYING FIBROUS EPULIS:**

**MARKWELL B.D. (1968)** <sup>[44]</sup> reported a case of painful Epulis in the lower left premolar region that was diagnosed as ossifying Fibrous Epulis and associated with TMJ Arthrosis presumably caused by reflex deviation of mandible to avoid biting the painful Epulis.

**ANDERSEN L, FEJERSKOR O, PHILIPSEN H P(1973)** <sup>[1]</sup> studied calcifying Fibroblastic Granuloma and described three characteristic zones on histopathology. They suggested that this may represent a stage in the development of Fibrous Epulis. This term has been proposed by Lee to indicate the non-neoplastic nature and principal histologic feature of a group of lesions peculiar to the gingiva.

**GARDNER D G (1982)** <sup>[28]</sup> clarified that peripheral odontogenic Fibroma and peripheral ossifying Fibroma do not represent the same lesion. The peripheral odontogenic Fibroma is an extraosseous counterpart of the central odontogenic fibroma and therefore should be referred to as peripheral odontogenic. fibroma (WHO). Peripheral Ossifying Fibroma is a reactive lesion and is known by other synonyms such as calcifying Fibrous Epulis etc.

**ORKIN D A, AMAIDAS V D (1984)** <sup>[55]</sup> reported a case of ossifying fibrous epulis occurring in a pregnant woman in both the anterior and posterior region of mandible causing displacement of left lower incisors.

**BUCHNER A, HANSEN L S(1987)** <sup>[14]</sup> analysed 207 cases of peripheral ossifying fibroma both clinically and histologically . They found that 60% of their lesions occurred in the maxilla, Incisor-cuspid region 50%, affecting females (1.7:1) in second decade more commonly. Histologically the lesions presented as ulcerated lesions with highly cellular fibroblastic connective tissue with dystrophic

calcification to non-ulcerated lesions with dense collagenised connective tissue with mineralized product in the form of bone and cementum like material. They proposed that the ulcerated and non-ulcerated lesions represent a spectrum of one lesion with different stages of maturation.

**BUCHNER A, FICCARA G, HANSEN L S(1987)** <sup>[13]</sup> reviewed and presented histomorphological spectrum of 9 cases of peripheral odontogenic Fibromas and they said all these lesions are to be considered as a spectrum of peripheral odontogenic Fibroma (WHO type) though they had a range of histological appearance. They also insisted that the term WHO type be used to distinguish them from peripheral ossifying Fibroma.

**PAUL AND MICHAELIDES (1992)** <sup>[46]</sup> When discussing the differential diagnosis of Peripheral Odontogenic Fibroma reported that peripheral odontogenic fibroma has to be differentiated from peripheral ossifying fibroma. The former had odontogenic epithelial islands and the latter dysplastic dentin. He also quoted that “Buchner & Hansen found a recurrence rate of 15.9% following excision in 207 cases of peripheral ossifying Fibroma whereas no recurrence were reported in their study in Peripheral Odontogenic Fibroma.

**YANG L J, JIN Y, DOI T, SEBINE I, OGAWAS K, MORI M (1993)** <sup>[78]</sup> elucidated the biological process of ossification and cemento ossification in calcifying Fibrous Epulis by means of immunohistochemical demonstration of BMP. They reported that the histogenesis of ossifying and cemento ossifying fibroma appear to be of two possible origins. Excessive proliferation of periodontal ligament and a metaplastic process occurring in the connective tissues fibres (non-periodontal ligament in origin) and the former is more common.

**SHAFFER W C, HINE M K, LEVY B M (1993)** <sup>[66]</sup> in their fourth edition of a textbook of oral pathology published that Peripheral Ossifying Fibroma also known as Peripheral Odontogenic Fibroma, Peripheral Cementifying Fibroma,

Calcifying / ossifying FibroidEpulis, Peripheral Fibroma with calcification should be surgically excised and submitted for microscopic examination for confirmation of diagnosis but they recur with some frequency and repeated recurrence is not uncommon. But in few studies a recurrence rate of 16% and 20% have been reported.

**NEVILLE B W, DAMM D D, ALLEN C M, BONQUOT J E (1995)** <sup>[51]</sup> in their textbook of oral and maxillofacial pathology have described that Peripheral Ossifying Fibroma occurs as a nodular mass, pedunculated / sessile that emanates from interdental papilla and occurs exclusively on Gingiva predominantly in teenagers and young adults with a peak prevalence in 10-19 years age group in females.

**CAWSON R A, BINNIE W H, SPEIGHT P, BARRETT A W, WRIGHT J M (1998)** <sup>[16]</sup> in their fifth edition of Luca's pathology of tumors of the oral tissues stated that Buchner A, Hansen Ls (1987) analysed Peripheral Ossifying Fibroma and its various patterns of calcification and ossification and emphasized that though some of these lesions were highly cellular and resembled an ossifying Fibroma microscopically they told that these were not a counterpart of the endosteal ossifying fibroma and were reactive in nature.

#### **DENTURE INDUCED HYPERPLASIA**

**BERNICK B.S (1948)** <sup>[7]</sup> studied the growths of Gingiva and palate reported that Denture injury tumor constituted the second largest group found on Gingiva and palate (11%) in their study and the continuous irritation first produced a chronic inflammatory reaction.

**ALEXANDER A.F. (1956)** <sup>[25]</sup> studied the allergic sensitization of the skin and oral mucosa to acrylic resin denture material and concluded that the methyl methacrylate liquid monomer is a sensitizer and can cause allergic, eczematous contact type of dermatitis and allergic stomatitis (sore month due to dentures).

**LOVE W D, GOSKA F A, MIXTOON R J(1967)** <sup>[40]</sup> studied the influence of non-systemic factors on the health of the denture supporting mucosa and proposed that fit of the denture had more influence on the condition of the supporting mucosa in addition to removal of denture at right age, Pt. Education

**BUDIZ E– JORGENSENA (1981)** <sup>[34]</sup> studied the lesions of oral mucosa associated with the wearing of removable dentures and reported that these may represent acute / chronic reactions to microbial denture plaque, a reaction to constituents of the denture base material or a mechanical denture injury. These include traumatic ulcers, allergic reactions to denture materials and acute infections under acute conditions. Chronic reactions include denture stomatitis caused by chronic infection or trauma, angular cheilitis and denture irritation hyperplasia, flabby ridges and oral carcinomas. Denture irritation hyperplasia is a common tissue reaction to ill fitting dentures, of the mucosa in contact with denture border (inflammatory hyperplasia, Epulis fissuratum, redundant tissue) and was found in 12% of denture wearers in their study.

**PRABHU S R, WILSON D.F, DAFFARY D.K., JOHNSON N.W (1993)** <sup>[58]</sup> in their textbook Oral diseases in tropics published that denture induced hyperplasias are usually located in the periphery of the denture, but may occur in any region where chronic physical irritation is present. Clinically they are present as elongated folds of tissue emanating from the base of the alveolar ridge and extending into the mucobuccal / mucolabial folds into which the flange of the denture fits.

**Histologically (Lutright DE 1974)** reported that there's an excessive amount of dense fibrous C.T with thick collagen fibre bundles, variable inflammatory cells, not very prominent vascularity and cellularity unless inflamed and focal areas of hyalinization and myxomatous degeneration. They

proposed that the distinction between localized hyperplastic lesions is largely on the basis of clinical and histopathological difference rather than on the basis of etiology.

**NEVILLE B W, DAMM D D, ALLEN C M, BONQUOT J E (1995)** <sup>[51]</sup> in their textbook of oral and maxillofacial pathology have reported that Epulis Fissuratum (Inflammatory Fibrous Hyperplasia. Denture Epulis, Denture induced tumour) occurs commonly on the facial aspect of gingiva although occasional cases have been reported in lingual aspect. They reported that the lesions occur more frequently in women, possibly because women tend to live longer, more women wear dentures, seek dental treatment more frequently and wear their dentures more frequently and for longer periods and post menopausal hormonal change may also play a role.

**CAWSON R A, BINNIE W H, SPEIGHT P, BARRETT A W, WRIGHT J M (1998)** <sup>[16]</sup> in their fifth edition of Luca's pathology of tumors of the oral tissues. Have reported that Bilateral symmetrical fibrous overgrowths of maxillary tuberosities which appear to be a developmental anomaly are structurally similar to denture induced hyperplasias.

**JOSEPH A.R. & JAMES J.S. (1999)** <sup>[63]</sup> They reported in their textbook Clinical Pathological Correlations that Denture Induced Hyperplasia is essentially the same process that leads to the traumatic fibroma, except that a denture is specifically identified as a causative agent and has also been designated as inflammatory hyperplasia, denture hyperplasia and epulis fissuratum.

**PHILIP S, EVERSOLE L R., WYSOCKI G P (2004)** <sup>[56]</sup> described inflammatory fibrous hyperplasia as proliferation of fibrous connective tissues with an associated chronic inflammatory response to injury. Ill fitting dentures with over extended flanges or older dentures that irritate vestibular tissue after alveolar ridge resorption may stimulate fibroblastic proliferation and collagen synthesis.

They are encountered in the maxillary / mandibular anterior vestibule where its associated with an ill fitting denture.

On microscopic examination they resemble scar tissue with decreased spindle shaped fibroblasts interposed between dense collagen fibres. When the fibrous hyperplasia extends into lip and BM, minor salivary gland lobules may be identified and will usually show acinar degeneration and ductal dilatation with inflammatory cell infiltrates (Chronic sclerosing sialadenitis)

### **TRAUMATIC FIBROMA**

**STONES H. H. (1962)** <sup>[68]</sup> Fibroma may arise from deep layers of the mucosa or from the periosteum of the jaws and even may arise from the Periodontal ligament membrane. They said that the terminal stage of an inflammatory hyperplasia may also resemble a fibroma. They reported a case of sublingual Fibroma on ventral side of tongue, which was pedunculated and hard. They classified fibromas as hard and soft fibromas. Hard fibromas contain thick interlacing bundles of collagen fibres and less vascular, soft fibromas are made vascular loosely arranged collagen fibres and when mucin is formed they are sometimes spoken as myxofibromas.

**REGEZI J A, COURTNEY R M, KERR D A, ARBOR A (1975)** <sup>[61]</sup> compared the fibrous lesions of the skin and mucous membrane which contain stellate and multinucleated cells. They reported that the various fibrous lesions of the skin and mucous membranes share the common histological feature of stellate and multi nucleated fibroblasts which are conspicuous under the light microscope because they contain a well developed rER which because of its increase RNA content stains basophilia and were apparent in about 1 % of the Irritation Fibroma. These lesions most likely represent a non-specific proliferation of the lamina propria / papillary dermis to various stimuli.

**SCHNEDIDER L C , WEISINGER E(1978)** <sup>[65]</sup> performed a histological review of 129 circumscribed mesenchymal lesions of the gingiva and reported that these includes peripheral giant cell reparative granuloma, peripheral ossifying fibroma, Inflammatory hyperplasia, Pyogenic Granuloma and hard fibromas (fibroepithelial Polyp) and 1 true fibroma is an extremely uncommon lesion.

**HOUSTON G D(1982)** <sup>[32]</sup> reviewed 464 cases of Giant cell fibromas and reported that they present clinically as asymptotic, papillary, pedunculated lesions commonly regarded as papilloma common in young persons, gingiva and histologically show both stellate type of giant cells with dendrite processes and multinucleated giant cells, few of them resembling Langhans giant cells and prominent vasculature and responds to simple surgical excision.

**BALOGH K, RICHARDSON J(1986)** <sup>[3]</sup> histochemically demonstrated the presence of oxytalan fibre and muco-polysaccharide and chondrogenesis in peripheral fibromas of gingiva. (cutright DE (1972) has reported osseous and chondromatous metaplasia under flabby ridges and Epulis fissuratum). They elucidated the histogenesis of cartilage and the cytological features suggested direct transformation from fibroblastic cells to chondrocytes and this evidence was supported by the presence of neutral and acid mucopolysaccharides in the loose stroma as well as in the ground substance of the cartilagenous center.

**SHAFER W G, HINE M K, LEVY B M(1993)** <sup>[66]</sup> in their fourth edition of a textbook of oral pathology published that Giant Cell Fibroma contain stellate and multinucleated giant cells occur not only in Giant Cell Fibroma but also in fibrous papule of the nose, ungual Fibroma, acral Fibrokeratoma, Fibroblastoma, Virus induced tumor of deer and retro cuspid papilla and pearly penile papul of glans penis. Fibroma because of the consistency fibroma may be either firm and resilient or soft and spongy, the clinical terms hard and soft fibroma were used but such

terms are of no significance and there is often little correlation between the consistency and the histological appearance of the lesion.

**ODELL E, LOCK C . LOMBARDI T (1994)** <sup>[52]</sup> studied the phenotypic characterization Giant Cell Fibroma and reported that these cells are positive for Vimentin and prolyl-4-hydroxylase indicating their fibroblast phenotype and proposed that these cells appear to be functional in collagen turnover.

**NEVILLE BW, DAMM D D, ALLEN C A, BONQUOT J E (1995)** <sup>[51]</sup> in their textbook of oral and maxillofacial pathology have described Irritation Fibroma / Traumatic Fibroma/ Focal Fibrous Hyperplasia / Fibrous Nodule is a true neoplasm / reactive hyperplasia of fibrous connective tissue in response to local irritation / trauma is doubtful. It microscopically contains nodular mass of dense collagenised connecting tissue which blends into surrounding normal connective tissue and is not encapsulated. They reported that Giant cell Fibroma differs from Irritatonal Fibroma clinicopathologically. They tend to occur in younger age group during the first three decades of life and microscopically contain loosely arranged vascular fibrous connective tissue.

**CAWSON R A, BINNIE W H, SPEIGHT P, BARRETT A W, WRIGHT J M (1998)** <sup>[16]</sup> in their fifth edition of Luca's pathology of tumors of the oral tissues. Have mentioned that Weathers DR. Callihan MD (1974) were the first to describe these lesions and found them to amount for 108 of 2000 oral fibrous nodules.

They reported that the giant cells appeared microscopically as stellate with large vesicular nuclei and dendritic processes and contains melanin granules. These giant cells were thought to be typical fibroblasts and these contain numerous intracellular micro fibrils and postulated a viral origin since it shared many features in common with virus induced fibroblastoma of deer. Immunohistochemical

demonstration of giant cells stained positively with vimentin and prolyl-A-hydroxylase indicating a functional fibroblastic phenotype.

**JOSEPH A.R. & JAMES J.S. (1999)** <sup>[63]</sup> They reported in their textbook *Clinical Pathological Correlations* that Peripheral Fibroma might be derived from the lamina propria of the submucosa or Periodontal ligament. They have described this as the gingival counterpart of the traumatic fibroma occurring in other mucosal regions. They proposed that in Giant Cell Fibroma, the multinucleated cells assume stellate shaped and they might be found in lesions throughout oral mucosa and occasionally on skin. Traumatic Fibroma: It is also known as irritation fibroma, focal fibrous hyperplasia and hyperplastic scar. It may be part of a rare Autosomal Dominant Syndrome known as Cowden's syndrome or multiple hamartoma syndromes presents with oral fibromas and papillomas, cutaneous papules, keratoses and Trichilemmomas benign and malignant neoplasms of the breast and thyroid and colonic Polyps.

### **FIBROEPITHELIAL POLYP**

**THOMA K. H (1960)** <sup>[73]</sup> proposed that Fibrous Epulis in early stages may appear as granulation tissue with some immature fibrous tissue, many inflammatory cells and large number of polymorphonuclearleucocytes infiltrate, indicating abscess formation. The lesion is sometimes called Granuloma Pyogenicum. With progression of the condition or as the source of irritation is removed more fibrous tissue is laid down, these collagen bundles are irregularly arranged. He proposed that the lesion that arises on the oral mucosa as a result of trauma presents a similar histological appearance and may be called a fibroepithelial polyp. If the source of irritation is removed, the histological picture becomes that of irregularly arranged bundles of collagen fibres with little vascularity and a well keratinised stratified squamous epithelial covering.

**PRABHU S R., WILSON D.F, DAFFARY D.K., JOHNSON N.W (1993)** <sup>[58]</sup> in their textbook Oral diseases in tropics published that In contrast to mucosal epithelium the connective tissue of oral mucosa is a heterogenous tissue made up of various cell and tissue components. The histopathology of oral mucosal lesions, which fall into the category of connective tissue hyperplasias is more complex compared to epithelial hyperplasias. These connective tissue lesions in oral mucosa in fact contain elements of both connective tissue and epithelial hyperplasia especially in fibroepithelial polyp, denture induced hyperplasia (epulis fissuratum) and papillary hyperplasia of palate. The pathobiology of the lesions frequently described as “Fibroepithelial Hyperplasia” indicates that both epithelium and connective tissue hyperplasia contribute to the lesions clinically. They published that there’s no evidence to suggest that the incidence of either generalised / focal oral mucosal hyperplasia is higher or lower in tropical countries. Fibroepithelial polyp are the most common of focal hyperplastic lesions of oral mucosa and common on trauma prone sites Buccal Mucosa, inner aspect of lip and tongue. Fibroepithelial polyp encountered on the gingiva is usually referred to as Fibrous epulis. It consists of mature fibrous connective tissue with variable cellularity and vascularity and variable distribution and intensity of chronic inflammatory cell infiltrate.

**NEVILLE B W, DAMM D D, ALLEN C M, BONQUOT J E (1995)** <sup>[51]</sup> in their textbook of oral and maxillofacial pathology have described Fibroepithelial polyp as fibrous hyperplasia occurring on the palate under a denture / leaf like denture fibroma which is a flattened pink mass attached to the palate by narrow stalk.

### **PERIPHERAL GIANT CELL GRANULOMA**

**THOMA K. H (1960)** <sup>[73]</sup> proposed that Giant Cell Epulis, Peripheral Osteoclastoma was called by a variety of names including myeloid epulis and

reparative peripheral osteoclastoma. They said that the pathogenesis may be an inflammatory hyperplasia of fibrous tissue in which the conditions are favourable for the formation of multinucleated giant cells and these giant cells have not been seen to divide and probably formed by fusion of other cells.

**GIANSANTI J S, WALDRON C A (1969)** <sup>[30]</sup> reviewed 720 cases of Peripheral Giant Cell granuloma and reported their clinical and histopathological features. They reported no predilection for location and any region of gingiva / alveolar mucosa may be involved.

**BHASKAR S N, DUANE AND CUTRIGHT, BEASLEY J D, PEREZ B (1971)** <sup>[8]</sup> studied 50 cases of Peripheral Giant Cell Reparative granuloma and reported their clinical and histological features.

**DAYAN D, BUCHNER A, SPIRER S (1990)** <sup>[24]</sup> analysed the mineralized products in Peripheral Giant Cell Granuloma and reported that the pattern of mineralisation is either woven or lamellar bone and dystrophic calcification. The most common type was the woven bone, which appeared alone or in combination with lamellar bone (in 82% of the lesions). Unlike Peripheral Ossifying Fibroma, no cementum like material was identified in Peripheral Giant Cell Granuloma. He also stated that Peripheral Giant Cell Granuloma originates from a response to intense irritation of the periosteum and that mononuclear stromal cells resemble latent proliferative osteoblasts (pre-osteoblasts) or osteoprogenitor cells.

**WILLIAM G SHAFER, MAYNARD K HINE, BARNET M LEVY (1993)** <sup>[66]</sup> in their fourth edition of a textbook of oral pathology published that Peripheral Giant Cell Granuloma also referred to as Giant cell Epulis, Giant cell tumor, osteoclastoma, microscopically contains delicate reticular and fibrillar connective tissue stroma with numerous giant cells which sometimes may be found within the lumina of these vessels.

They also quoted on histochemical observation of giant cell granuloma found distinct differences in the distribution of tyrosine and sulphhydryl groups in different multinucleated giant cells. Few authors suggested that the giant cells might have aroused through fusion of endothelial cells and suggested it to be a malignant metastasizing one.

**BADNER L, PEIST M, GATOT A, FLISS D M, SHEVA B (1997)** <sup>[10]</sup> investigated the association of demographic, systemic health and oral health characteristic with large (72 cm) Peripheral Giant Cell Granuloma and assessed its growth potential and the possible underlying causes and reported that large lesions are more common in women with poor oral hygiene and xerostomia.

**JOSEPH A.R. & JAMES J.S. (1999)** <sup>[63]</sup> reported in their textbook Clinical Pathological Correlations that Peripheral Giant Cell Granuloma is a basically hyperplastic granulation tissue with scattered multinucleated giant cells that are shown to be derived from macrophages by ultrastructural studies and they reported that ultrastructurally fusion of plasma membrane of adjacent macrophages were demonstrated and immunohistochemically macrophages and giant cells share similar antigenic markers, such as muramidase and  $\alpha$ -antichymotrypsin. They also showed that these giant cells appear to be non functional in the usual sense of phagocytosis and bone resorption.

## **SUMMARY AND CONCLUSION**

We studied the clinicopathological correlation of focal reactive overgrowths of oral mucosa for a period of 2 years from 2002 – 2004 and the distribution of various connective tissue fibres were analysed in 55 cases.

### **OBSERVATIONS IN CLINICO PATHOLOGICAL STUDY:**

1. Histopathological confirmation of reactive lesions is essential because clinically these lesions have a similar picture and microscopic confirmation is mandatory.
2. Fibrous epulis were the most common lesion followed by Pyogenic granuloma, Calcifying fibrous epulis, Fibro epithelial polyp, Traumatic fibroma, Denture induced hyperplasia and Peripheral giant cell granuloma.
3. The M: F ratio of localized reactive over growths of oral mucosa is 1:1.6 which indicates a predominant female incidence.
4. Majority of these lesions presented as painless over growths with shorter duration. Most of them were sessile, firm, pink coloured, irregular lesions with smooth surface.
5. Gingiva is the most common site followed by buccal mucosa, palate, tongue and lip.
6. Histopathology revealed that most of the lesions were covered by hyperplastic, parakeratinised stratified squamous epithelium with branching rete pegs and majority were less cellular with increased vascularity and inflammatory cell infiltration. A direct correlation between vascularity and inflammatory cell infiltration was noted.

#### **OBSERVATIONS IN THE STUDY OF “THE DISTRIBUTION OF CONNECTIVE TISSUE FIBRES”:**

1. Collagen fibres were the predominant fibre group in the Fibrous epulis, Calcifying fibrous epulis, Traumatic fibroma, Denture induced hyperplasia and Peripheral giant cell granuloma.  
These were present as thick fibres in Traumatic fibroma and Fibrous epulis. Thin fibres were predominantly present in Peripheral giant cell granuloma and Calcifying fibrous epulis. It was observed that in areas of

inflammation thin collagen fibres were present. Mixed fibres were observed in Denture induced hyperplasia.

2. Elastic fibres were present in Traumatic fibroma, Fibrous epulis and Calcifying fibrous epulis.
3. Reticular fibres were predominantly present in Pyogenic granuloma, Peripheral giant cell granuloma and Calcifying fibrous epulis.

Thus it was observed in our study that Collagen fibres were the most prominent fibre group present in the Reactive Fibrous Overgrowths of Oral mucosa followed by equal distribution of elastic and reticular fibres.

Our study gives an overview of connective tissue fibers distribution in various Focal Reactive Overgrowths of oral mucosa. A detailed study with increased sample size will throw some light in the clear understanding of the behaviour of these lesions.

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