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Immunohistochemical and transmission electron microscopy study regarding myofibroblasts in fibroinflammatory epulis and giant cell peripheral granuloma

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Abstract

Fibroblasts represent the main cellular population in the connective tissue; they have a central role in extracellular matrix (ECM) synthesis, degradation and remodeling. These cells may express a substantial heterogeneity regarding their morphology and functions in pathological conditions and during tissue remodeling. Myofibroblasts are a good example for heterogeneity and phenotypical changes. These cells can be morphologically and immunologically defined by the expression of specific cytoskeleton proteins. Myofibroblasts show cytoplasmic actin microfilaments organized as stress fibers and interconnected by gap or adherens junctions. These cells come also in contact with extracellular matrix by focal contacts. Myofibroblasts play fundamental roles in pathologic conditions, even by activation and proliferation or by deletion. Moreover, these cells seem to be involved in formation and repair of the ECM compounds, proliferation and differentiation of the epithelial, vascular or neurogenic elements. The purpose of the present study is to emphasize the presence and distribution of myofibroblasts in the reactive stromal tissue of granulation tumors in the oral area, fibroinflammatory epulis and giant cells peripheral granuloma, by means of immunocytochemical and transmission electron microscopy studies. Both tumor types shown a common characteristic of the presence of reactive inflammatory stromal tissue and myofibroblasts are a common issue.

Keywords: α -SMA, fibroinflammatory epulis, giant cells, myofibroblasts.

Introduction

Fibroblasts that play an important role in wound healing and are also involved in a wide range of fibroconnective diseases are known as myofibroblasts. They are cells with an intermediate status between fibroblasts and smooth muscle cells, thus some authors named them smooth muscle like cells [1, 2].

Cell motility, proliferation, differentiation, apoptosis, morphogenesis, tissue repair, inflammation, and the immune response are initiated, maintained, and terminated by local interactions between cells. These interactions are brought about by contact of cells with each other or with the extracellular matrix and response to soluble mediators [3]. These cells were identified morphologically like a family of paracrine cells that play an important role in the regulation of these fundamental processes [4].

Immunohistochemical characterization (PAP technique) of myofibroblasts is based on antibody reactions to two of three filament systems. These three systems are composed of actin, a component of the microfilaments; vimentin, desmin and laminin, members of the intermediate filament system;

the tubulins of the microtubules. The β - and γ -actins are expressed by all cells, including myofibroblasts, which may also express α -smooth muscle actin (α -SMA).

Myofibroblasts show cytoplasmic actin filaments as stress fibers and are interconnected by gap or adherens junctions. Actin is the most abundant protein in the myofibroblast cytoskeleton and polymerizes as filaments. Actin filaments are present especially beneath the membrane where they form a network that represent the mechanical support that induces the shape and cell motility [5].

In some pathological conditions of the oral mucosa or of the mandible, myofibroblasts can be found in benign epithelial tumors of the maxillary or of the jaw, in nodular fasciitis, malignant fibrous histiocytoma or benign tumors and hyperplasia in soft orofacial tissues. In the latter group, we can include the fibroinflammatory epulis and giant cell peripheral granuloma [6, 7]. It can be considered that they represent a reactive inflammatory disease in which myofibroblasts could appear. Fibroinflammatory epulis is a inflammatory reactive disease to chronic irritative factors or trauma. The inflammatory reaction is associated with a proliferative vascular reaction, immature fibrous tissue and

also abundant myofibroblasts with similar characters with those in giant cell peripheral granuloma [8, 9].

The purpose of the present study is to emphasize the presence, distribution and the ultrastructure of the myofibroblasts in granulation tumors developed in gingival fibromucosa.

☐ Material and Methods

We used tissue samples from nine patients diagnosed with fibroinflammatory epulis and giant cell peripheral granuloma. Patients were selected from those admitted in Oral and Maxillo-Facial Surgery Clinic in "St. Spiridon" University Hospital in Iassy. Tumors were located in the maxillary or mandibular gingival fibromucosa with an oral and vestibular progression.

Samples for immunocytochemistry PAP-technique were processed for paraffin inclusion after fixing in neutral 10% formaldehyde. Paraffin was removed and sections hydrated in PBS. Peroxydase block was performed with 30% peroxide in distilled water. Incubation was performed overnight at 4°C with 1:50 dilutions of mouse anti-human vimentin and mouse anti-human alpha-actin (Santa Cruz Biotech). After primary antibody removal, sections were washed three times in PBS for 5 minutes and then incubated with secondary biotinilated antibodies (Dako Cytomation LSAB2 kit-HRP). Reaction development was performed by DAKO 3% DAB solution. Nuclear counterstaining was performed by Mayer's Hematoxylin (DAKO) for 1 minute. After washing and xylene clarification, the slides were examined in a brightfield Olympus BX40 microscope. Samples for electron microscopy were processed following the standard protocol for TEM: double fixing in glutaraldehyde and osmium tetroxide, washing in buffer solution, dehydration by alcohol and acetone, epon-embedding and ultramicrotome sectioning. Ultrafine sections were deposited on copper grids of 300 MESH, contrasted by lead citrate and uranyl-acetate, and then examined in a Philips CM 100 transmission electron microscope.

☐ Results

For both tumor types, we have observed in the epithelia deep ulceration areas and granulation tissue

alternating with areas of epithelia parakeratinisation. In the superficial chorion, the inflammatory reaction dissects collagen fibers. In the deep chorion, sclerosis areas are characterized by the presence of a hematic infiltrate together with a polymorph infiltrate, dissected by collagen fibers.

The present study shows a disposition comparable for the two tumor types α -actin staining was positive for myofibroblasts in the deep chorion (Figure 1).

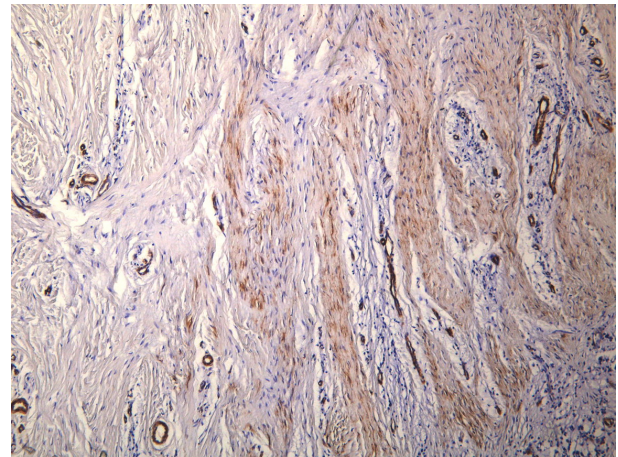


Figure 1 – α -Actin-positive cells in medium vessel walls and in the disseminated myofibroblasts (IHC, $\times 20$).

At this level, we have a grouped distribution for these cells. Another myofibroblast characteristic is represented by their tendency to migrate toward the inflammatory region. In the tumor, we have observed an irregular distribution of myofibroblasts.

In the deep chorion, the immunostaining for α -SMA was intensely positive in submucosa, while in the mucosa we have few staining except capillary walls (Figure 2, a and b). The cytoskeleton marker was present in the muscular element of medium vessels (Figure 3).

In granulation tissue or in pathological fibrous conjunctive tissue, fibroblasts gain new morphological characteristics. In the cytoplasm is developed a fibrillar system, which contains parallel bundles of fibers alike those from the smooth muscle cells level, extended along the axis of the cell (Figure 4).

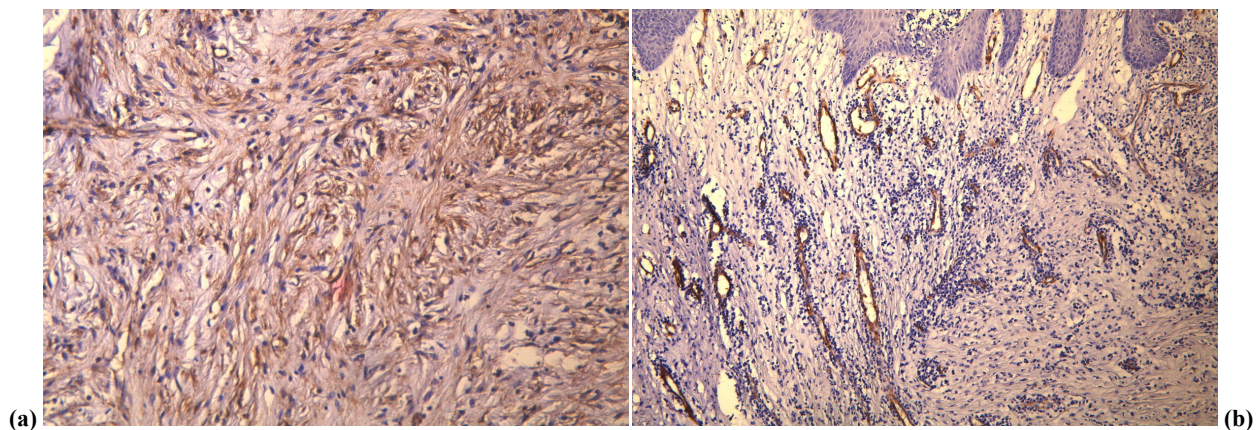


Figure 2 – (a) Abundant myofibroblasts in deep chorion, positive for α -actin (IHC, $\times 20$). (b) α -actin-positive cells in the vascular walls (IHC, $\times 10$).

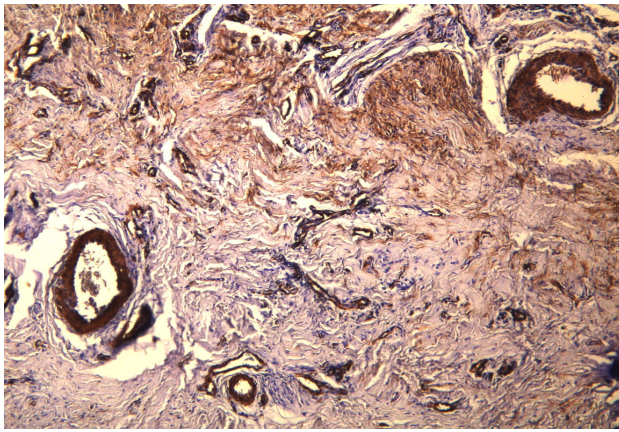


Figure 3 – α -Actin-positive myofibroblasts near the inflammatory process and in medium vessel walls (IHC, $\times 10$).

Between the fibrils bundles and under membrane could be observed electron-opaque areas similar to focal adhesion junctions, characteristic to smooth muscle fibers (Figure 5).

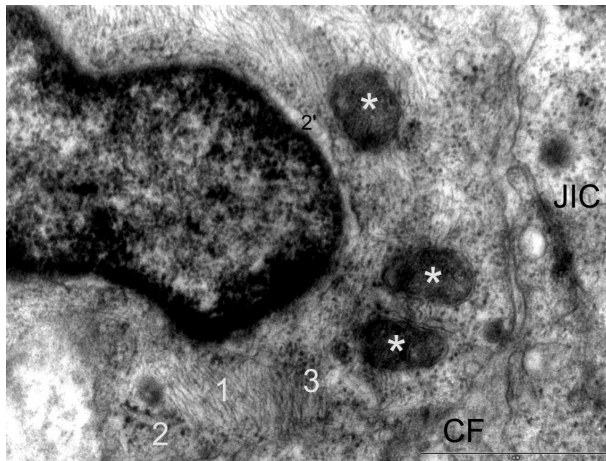


Figure 5 – Myofibroblast in detail of the perinuclear region with dense actin filaments localized in the neighborhood of the nucleus establishing focal contacts with extracellular matrix (CF) and intercellular contacts (JIC). In the cytoplasm are remarked a lot of mitochondria (*) and RER zones (2) and free ribosomes (3) perinuclear situated, all these organelles being involved in synthetic and cellular secretion activity. Perinuclear is remarked vacuolization of RER, sign of an intense synthetic activity (2') (TEM, $\times 40000$).

Even those fibrillar structures occupy the most part of the cell; the rest of the cytoplasm contains compact cisterns of rough endoplasmic reticulum, characteristic for normal fibroblasts (Figure 6).

Nuclei present multiple indentations or more profound plies (Figure 7). In addition, there are present many intercellular connections structured as desmosome-like structure.

We observed that in fibroinflammatory epulis, there is an inflammatory reaction associated with a vascular proliferation reaction.

As it can be seen in (Figure 8), peripheral granuloma is abundant in giant cells with multiple nuclei. The presence of the mastocytes is usual for both

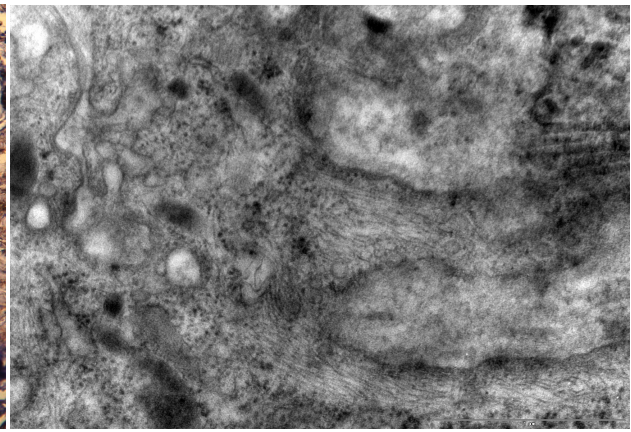


Figure 4 – Myofibroblast, intracytoplasmic actin filaments, disposed subplasmalemmal in stress fibers (TEM, $\times 40000$).

infiltrate types. Myofibroblasts present similar characteristics to both tumor types (Figures 9 and 10).

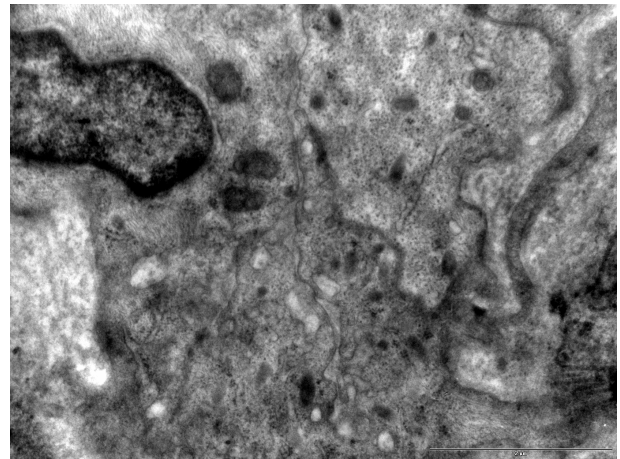


Figure 6 – Myofibroblast, actin filaments sectioned transversal and collagen fibers: detail. These cells are in contact also with extracellular matrix by focal contacts, a transmembrane complex composed by intracellular contractile microfilaments and by fibronectin as a participant from the extracellular medium (TEM, $\times 20000$).

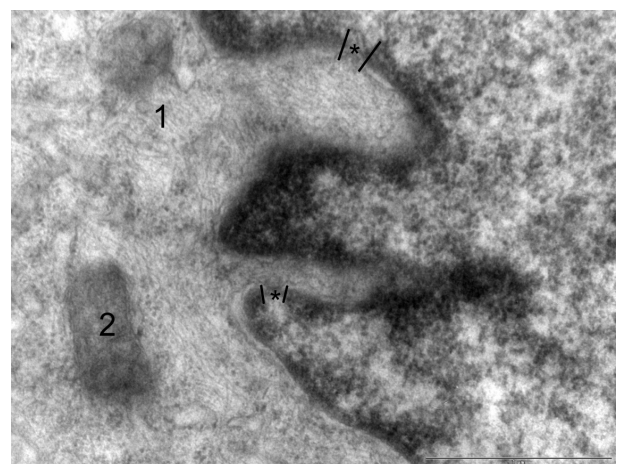


Figure 7 – Myofibroblast, perinuclear detail, actin filaments (1) mitochondrion (2), nucleus, actin filaments prominence in the formed spaces. Dilated nuclear pores (*), sign of an intense translation activity (TEM, $\times 40000$).

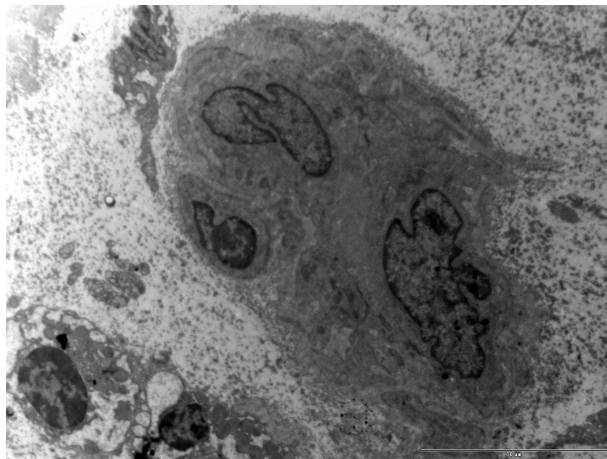


Figure 8 – Multinucleate giant cell: ML and lymphocytes (TEM, $\times 40000$).

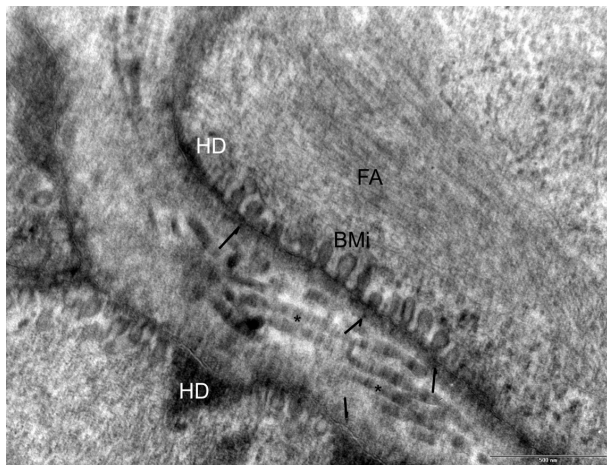


Figure 9 – Epithelio-conjunctive contact zone, epithelial cells and superficial intercellular chorion with collagen fibers (*) and hemidesmosome junction (HD), near anchoring plaques from collagen IV (arrows). Actin filaments (FA) converge to HD and basal membrane plies (MBi) are uprights (TEM, $\times 50000$).

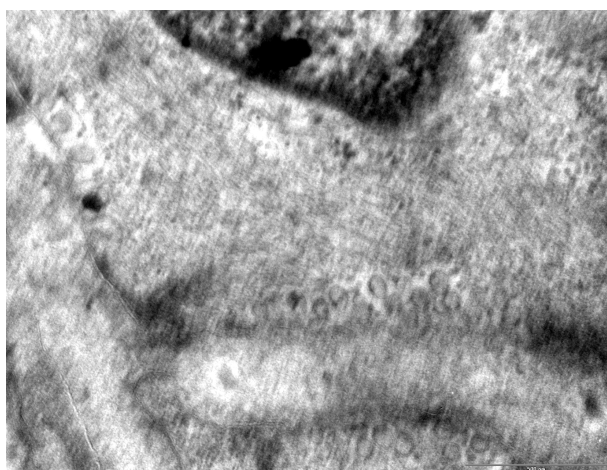


Figure 10 – Epithelio-conjunctive contact zone (detail, hemidesmosome) (TEM, $\times 60000$).

It is remarkable the benignity character of each tumor, which is evidenced by the integrity of the intercellular junctions or the cell-extracellular matrix junctions (Figure 11).

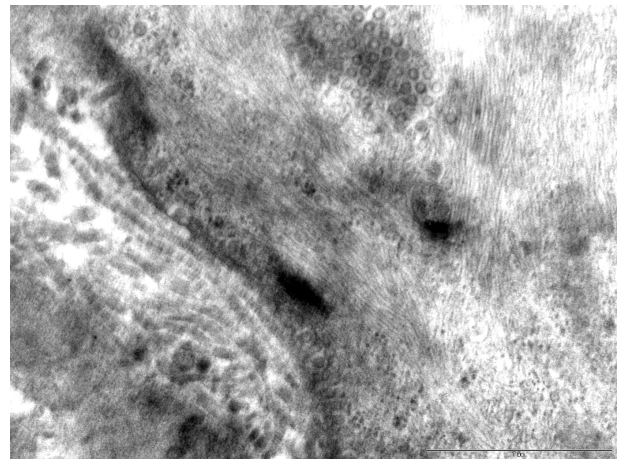


Figure 11 – Myofibroblast, intracellular stress fibers, extracellular collagen. Comparative aspect between intracellular stress fibers and those of extracellular collagen; can be observed the absence of actin filaments periodicity (TEM, $\times 60000$).

Discussion

Myofibroblasts may be defined morphologically and immunologically through identification of expressed cytoskeletal protein. Vimentin, desmin and α -SMA are the three filaments most often used to classify myofibroblasts. Expression of these proteins may vary with the tissue studied whether the cells are studied in situ or in culture and, even within a given tissue, whether the cells are activated by hormonal or cytokine treatment or by disease. Based on immunohistochemical staining of these filaments in a given tissue, a classification system has been proposed. Myofibroblasts that express only vimentin (V-type), those that express vimentin and desmin (VD-type), those that express vimentin, α -SMA and desmin (VAD-type), those that express vimentin and α -SMA (VA-type) and those that express vimentin and myosin (VM-type) [10–12].

In the two tumor types investigated by the usage of α -actin cytoskeletal marker, we have remarked an increased fibroblastic activity. Myofibroblasts are present in the lesion isolated or grouped, near and toward the inflammatory process. We have shown that the expression of α -SMA is highly correlated with myofibroblasts in the granulation tissue on the fibroinflammatory epulis and giant cell peripheral granuloma.

Lymphoplasmacyte infiltrate is located in the superficial chorion while in the deep chorion the fibrous sclerosis dominates. Epithelial changes have a reactive character by papillomatous proliferation, with long crysts, superficial parakeratosis, and sometimes, subject to linear atrophy or ulceration, replaced by granulomatous inflammatory processes. Fibroblasts show a perivascular placement and progress toward endothelium.

Lesion distribution does not depend on chorion disposition, being present either in the superficial or deep areas. In fibroinflammatory epulis, the lymphoplasmacyte infiltrate is located in the superficial chorion while in the deep chorion the fibrous sclerosis

dominates. The difference between the two tumor types is represented by the giant multinucleated cells that were better shown by electron microscopy [12].

In the present study, electron microscopy shows the presence of intracytoplasmic actin filaments, proof of phenotypical changes fibroblast–myofibroblast, in the samples from the investigated cases.

The cytoplasm develops a fibrillar system that contains bundles of parallel fibers seem-like with those into the smooth muscle cells, aligned along the cell axis. Among the fibril bundles and under the membrane we have observed electron-opaque areas similar to focal adhesion junctions and specific for the smooth muscle cells. The nuclear pores are enlarged, and that indicates an intense activity while perinuclear the dense actin filaments establish links with the ECM by focal contacts; focal contacts are a transmembrane complex formed by intracellular contractile elements and fibronectin from the ECM part. In epulis like lesions all connective elements are involved (vessels, cells, fibers), while epithelial elements show only reactive changes (specific for benign tumors) [13].

As cells identified by transmission electron microscopy in the granulation tissue in benign tumors have been interpreted as modified fibroblasts that express α -SMA. Intercellular connections are desmosome-like.

Perinuclear, the RER vacuolization is a sign of intense synthetic activity while in the cytoplasm we can see numerous mitochondria, RER and free ribosomes around the nucleus. All these aspects suggest an intense synthetic secretion activity of the cell.

Reactive tissue around the peripheral granuloma is rich in giant cells with multiple nuclei and possible phagocytic activity. Myofibroblasts show similar characteristic in both tumor types [6, 7].

The integrity of intercellular junctions or of the cell-matrix junctions indicates the benign character of both tumors [7, 14].

By cytoskeletal proteins, α -SMA, myofibroblasts in fibroinflammatory epulis and giant cell peripheral granuloma modulate and regulate the local accumulation of connective tissue cells and extracellular material (stromal reaction).

☐ Conclusions

Both types of benign connective tissue tumors: fibro-inflammatory epulis and giant cell epulis (giant cell peripheral granuloma) show that myofibroblasts are present in both tumors, isolated or grouped, near and toward the inflammatory process. α -Actin-immuno-staining was positive for myofibroblasts in deep chorion, in the smooth muscle cells in medium vessels and for the myofibroblasts near the inflammatory process.

The lymphoplasmocyte infiltrate is located in the superficial chorion while in the deep chorion the fibrous sclerosis dominates.

In giant cell epulis, the inflammatory infiltrate show multinuclear giant cells shown by transmission electron microscopy.

Lesion distribution depends on chorion localization, involving either the superficial or the deep region. In the investigated lesions, all connective elements participated.

Modulation of phenotypic trans-differentiation of fibroblasts is dependent on a complex micromedium branched in a network where the growth factors, adhesion molecules and the components of the extracellular matrix are active by involved.

Electron microscopy studies confirm that the myofibroblast are present isolated or grouped, in the neighborhood and to the inflammatory process.

Also, it can be observed intracytoplasmic actin filaments presence, as a proof of the phenotypic modification of the fibroblast–myofibroblast, in both anatomopathological types.

Myofibroblasts situated at the same level have an intense activity of the synthetic and secretors organelle. These cells secrete a large amount of collagen (type I and III). In the inflammatory superficial and profound zones, it is a participation of the macrophage cells, neutrophils and mastocytes.

In the lesional zone, the most important cells are activated fibroblast and myofibroblast. As a particularity, it is remarked that the myofibroblasts are perivascular positioned and reached endothelia. About the lesions distribution, this takes in consideration the chorion territory, focusing the superficial or even the profound zone. In the epulis lesions all the elements of the conjunctive component (vessels, cells, fibers) participate, superjacent epithelial modifications have a strict reactive character (the particular aspect of the benign tumors).

Actin filaments localized in the neighborhood of the nucleus established supermature focal contacts with extracellular matrix and intercellular contacts with neighborhood cells.

In the contact epithelial-conjunctive zone, junctions as hemidesmosomes and basal membrane plies are signs of the lesional benignity.

Many oral epithelial tumors are characterized by local accumulation of conjunctive cells and extracellular matrix, these processes being known as reactive stroma.

The identification and quantification of contractile specific compounds of the myofibroblasts may represent the key for evaluation of tumor prognosis and treatment issues in maxillofacial specific benign pathology.

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