# Myofibroblasts in Oral Health and Disease

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## ABSTRACT

Myofibroblasts (MFs) are the cells that are not only essential for the integrity of the human body by virtue of its role in physiological tissue repair (wound-healing), but can also threaten it by its ability to promote tumour development. Under physiological conditions, after wound healing, MFs disappear by apoptosis, but when there is continued insult, these myofibroblasts persist in the tissue and result in dysfunctional repair mechanisms causing excessive secretion of extracellular matrix with resultant fibrosis and scarring. Myofibroblasts are phenotypically altered fibroblasts and are a unique group of smooth-muscle like fibroblasts that have a similar appearance and function regardless of their tissue of residence. Myofibroblasts originate from different precursor cells, the major contribution being from local recruitment of connective tissue fibroblasts. However, local mesenchymal stem cells, bone marrow-derived mesenchymal stem cells and cells derived from an epithelial-mesenchymal transition process, may represent alternative sources of myofibroblasts when local fibroblasts are not able to satisfy the requirement for these cells during repair.

Apart from pathological remodelling of tissues, they play an important role in organogenesis and oncogenesis, inflammation, repair, and fibrosis. Because of their ubiquitous presence in all tissues, MFs play important roles in various organ diseases and perhaps in multisystem diseases as well. In the light of such severe consequences of MF appearance and dysfunction, the necessity of more profoundly understanding the molecular mechanisms of MF formation and function is essential. This paper highlights the overview of myofibroblasts, and their role in health and disease particularly in relation to diseases of oral cavity.

## Introduction

Myofibroblasts, by simple definition, are specialised fibroblasts, with smooth like features characterised by presence of contractile apparatus.[1] They are unique cells and are essential for the integrity of the mammalian body by virtue of its role in wound healing, but it can also threaten it by its ability to promote tumor development. Through the secretion of inflammatory and anti inflammatory cytokines, chemokines, growth factors, as well as extra cellular matrix proteins and proteases, they play an important role in organogenesis and oncogenesis, inflammation, repair and fibrosis in most organs and tissues. It is an almost universal cellular component in mammalian lesions, but not a typical component of normal untraumatised tissues.

The concept of Collagen, being the main element responsible for contraction of wound, changed in 1950. It was discovered that specialised fibroblasts were present in the granulation tissues. Microscopic studies revealed that these specialised cells are similar to that of smooth muscle cells which are capable of contraction. Later these smooth muscle like cells are termed as Myofibroblasts, by Gabbiani in 1971.[2]

Partly because of its absence in normal tissues, it has not been a part of conventional histologic teaching and has contributed difficulties in explaining the nature of these ubiquitous cells and in defining it. This article reviews on some important hallmarks related to its structure, immunophenotypes, origin and fate, its role in normal and in pathologic situations.

## Structure

Myofibroblasts have several unique morphological characteristics, few of which are present in fibroblast as well as smooth muscle cells. They are spindle shaped cells (Fig 1) with numerous cytoplasmic extensions containing actin microfilaments called as stress fibres and they are connected to each other by adherens and gap junctions and are connected to extracellular matrix by a transmembrane complex known as fibronexus.[3]

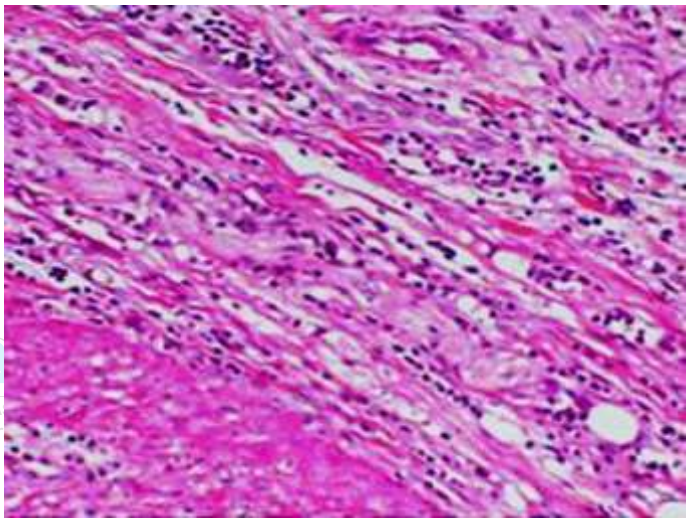

*Fig 1: Myofibroblasts in stromal cells (Courtesy: Yuhiko Fuyuhiro, Masakazu Yashiro, Satoru Noda, Shinichiro Kashiwagi, Junko Matsuoka, Yosuke Doi, Yukihiro Kato, Kazuya Muguruma, Tetsuji Sawada, Kosei Hirakawa. Myofibroblasts are associated with the progression of scirrhous gastric carcinoma. Journal of Experimental and Therapeutic Medicine; July 2010, 547-551)*

Transmission electron microscopy shows, the cell membranes displays numerous invaginations. The cytoplasm is rich in well developed RER, Golgi apparatus, mitochondria and intended nucleus. (Fig 2)

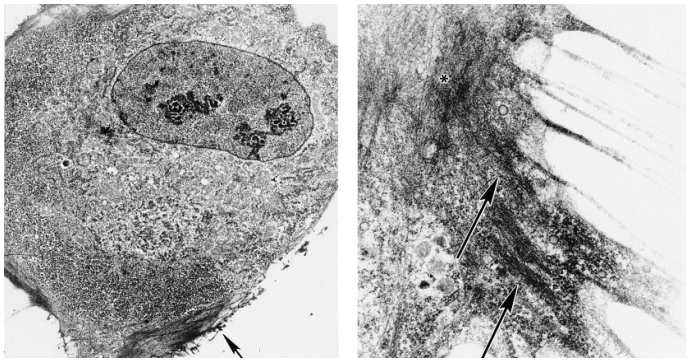

*Fig 2: Transmission electron microscopy showing tubular epithelial-myofibroblast transdifferentiation (TEMT) (i) A cell in the eary stage of transformation shows hypertrophy, an elongated morphology and a loss of apical polarity, micrvilli and tight junctions.*

*There are prominent bundles of actin microfilament lying peripheral to the cytoplasm (arrow head) (ii) A cell at the alte stage of transformation showing characteristic actin microfilaments bundles (arrows) throughout the cytoplasm. Magnification (i) x4200 (ii) 18,300*
*(Courtesy: Jun-Ming Fan, Yee-Yung Ng, Prudence A Hill, David J Nikolic-Paterson, Wei Mu, Robert C Atkins and Hui Y Lan. Transforming growth factor- β regulates tubular epithelial-myofibroblast transdifferentiation in vitro. Journal of international society of Nephrology (1996) 56, 1455 – 1467)*

Since, Myofibroblasts share features with fibroblasts (RER and Golgi apparatus) and with smooth muscle cells (myofilaments), it is seen that MF's are devoid of lamina – a structure seen in smooth muscle cells.

Further, α-smooth muscle actin (α-SMA) is present in others cells other than myofibroblasts like pericytes, endothelial cells, myoepithelium and pathological epithelia and one should not rely only on expression of α-SMA alone in identification or in distinguishing myofibroblasts. So, electron microscopy plays an important role in distinguishing myofibroblasts from other cells.

## Immunphenotypes

Almost all myofibroblasts express α-SMA, an actin isoform present in most of the types of smooth muscle cells. It is considered to be the main IHC marker in identifying myofibroblasts.[4]

Apart from α-SMA, they also express desmin, myosin and vimentin.(Fig 3) Based on these expressions, myofibroblasts disclose five immunophenotypes: a) phenotype V, cells expressing vimentin alone; b) phenotype VA, those expressing vimentin and α-SMA; c) phenotype VD, those expressing vimentin and desmin; d) phenotype VAD, those expressing vimentin, α-SMA and desmin and e) phenotype VAM, those cells expressing vimentin, α-SMA and myosin.

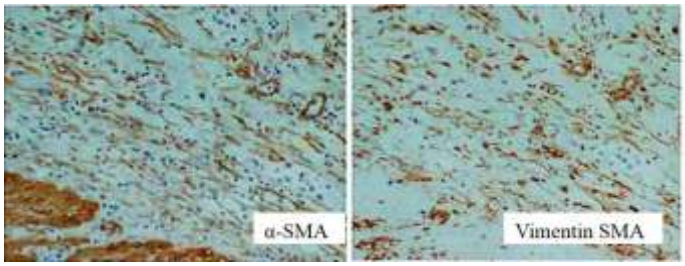


*Fig 3: Myofibroblasts expression in stromal cells (Courtesy: Yuhiko Fuyuhiro, Masakazu Yashiro, Satoru Noda, Shinichiro Kashiwagi, Junko Matsuoka, Yosuke Doi, Yukihiro Kato, Kazuya Muguruma, Tetsuji Sawada, Kosei Hirakawa. Myofibroblasts are associated with the progression of scirrhous gastric carcinoma. Journal of Experimental and Therapeutic Medicine; July 2010, 547-551)*

## Origin, differentiation of myofibroblast

Their occurrence in various physiological and pathological situations, makes it difficult to think about its exact source of origin. It is uncertain that the origin of myofibroblast is from the progenitor stem cells (possibly neuroepithelial stem cells), from the neural crest or simply trans-differentiate from the resident tissue fibroblasts or from tissue smooth muscle cells.[5]

In normal conditions, fibroblastic cells exhibit few or no actin associated cell to cell and cell to matrix contacts and little ECM production.[6]

After tissue injury, they become activated to migrate into the damaged tissue and to synthesize ECM components[7] by cytokines locally released from the inflammatory and resident cells[8]. Another important stimulus for this transition is the change of the mechanical microenvironment. In response to these mechanical challenge, fibroblasts gain contractile stress fibers that are composed of cytoplasmic actins,[6] hallmarking them as "Protomyfibroblats".

The term protomyofibroblast are termed for those fibroblasts with stress fibers that do not express α-SMA. For the transformation of protomyofibroblasts into mature myofibroblasts, mechanical stresses along with certain cytokines are necessary.

## Cytokines necessary for myofibroblast differentiation

Various cytokines and growth factors have a roles in myofibroblast differentiation[8]. Among these, especially, the transforming growth factor (TGF) –β1, (Fig 4) is the major growth factor and a potent inducer of myofibroblastic differentiation[9].

Platelet derived growth factors (PDGF) plays an important role in the differentiation of fibroblasts into protomyofibroblasts[10]. TGF –β1 and ED – A FN (a variant of fibronectin) are key players in differentiation of protomyfibroblasts into mature myofibroblasts. Other factors like Granulocyte-macrophage colony stimulating factor (GM-CSF) and integrins also play a role.[10]

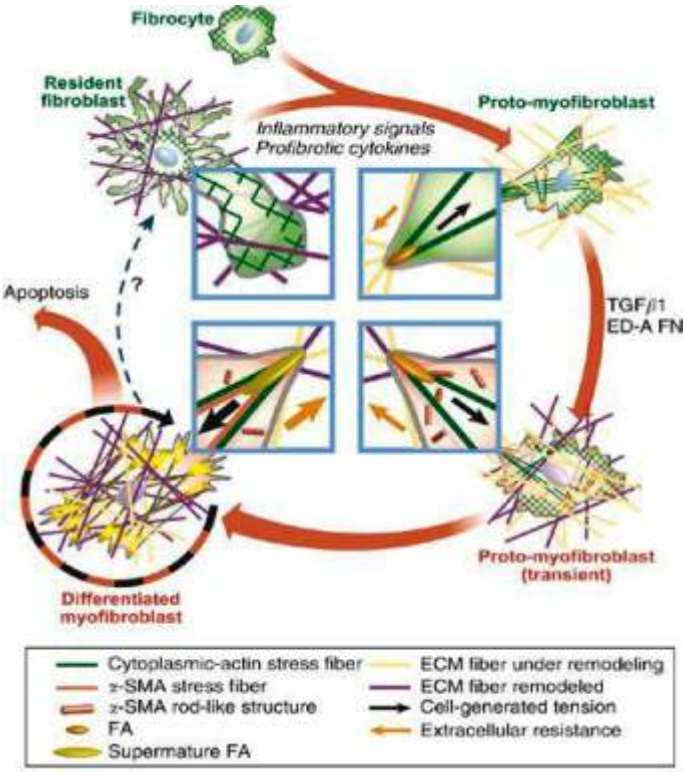


*Fig 4: Differentiation of fibroblast into Myofibroblast. (Courtesy: Hinz Boris. Formation and Function of the Myofibroblast during Tissue Repair. J Invest Derm. 2007;127:526–537)*

## Myofibroblasts in physiological situations Morphogenesis and organogenesis

Through epithelial-mesenchymal interactions, myofibroblasts are the main components of morphogenesis and organogenesis.[5] They do so by the discharge of soluble mediators of inflammation and growth factors and expression of their receptors and by the production of interstitial matrix and molecules of basement membrane.[5]

## Normal wound healing and wound contraction

Immediately after injury, the healing process allowing to restoration of the injured tissue occurs. According to the morphological changes in the course of the healing process, there occurs three phases of events described as a) Inflammatory phase, b) proliferative phase, for the development of granulation tissue and c) regenerative phase for maturation, scar formation and reepithelialisation[11].

Myofibroblasts appear to be key cells in the process of wound healing and are found more numerous in the exudates layer of granulation tissue. Prostaglandins synthesized by these cells promote healing by restoration of the epithelium. Contraction of wound is because of the presence of α-SMA filaments in the cytoplasm of these cells.[12]

The ECM, which is a mixture of collagen and ground substances and enzymes like matrix metalloproteinases (MMP's), required for tissue remodelling are also secreted by these cells.

So, they play a key role in the wound healing, seemingly as an addition to their function in normal growth and differentiation.[5]

## Myofibroblasts in pathological situations Role in inflammatory conditions

Myofibroblasts have an important position in the inflammatory response.[5] They produce both cytokines and chemokines and are capable of augmenting or down regulating the inflammatory response by the secretion of soluble mediators of inflammation. They also synthesise prostaglandins, expressing both COX-1 and the inducible COX-2 protein. On activation, myofibroblasts also express molecules for adhesion like intracellular adhesion molecule-1, vascular adhesion molecule and neural cell adhesion molecule. Thus lymphocytes, mast cells and neutrophils may dock on the myofibroblasts and participate in organised immunological and inflammatory reactions.

Ultrastructural study of various lesions of the oral cavity like giant cell fibroma[13] and Phenytoin induced gingival hyperplasia[14] revealed cells with numerous intracytoplasmic myofilaments with electron dense bodies similar to smooth muscle cells and fibroblasts and these cells are referred to as myobfbroblasts.

## Myofibroblasts in Odontogenic cysts and tumors

Presence of myofibroblasts has been reported in the stroma of odontogenic cysts and tumors. Electron microscopic studies have demonstrated the presence of myofibroblasts in the stromal component of ameloblastoma (Fig 5e and f and has been proposed that the presence of these cells could contribute to its aggressive behaviour[15].

Various studies have been conducted on odontogenic cysts and tumors and has revealed that myofibroblasts were particularly more in lesions with locally aggressive behaviour like odontogenic keratocyst and solid variant of ameloblastoma.[16]

Studies conducted using IHC to demonstrate α-SMA in odontogenic cysts reported that a variable proportion of the cyst wall fibroblasts showed expression for α-SMA. The results of these study demonstrated that myofibroblasts contribute to cyst wall elasticity and also helps in cyst wall expansion.[17]

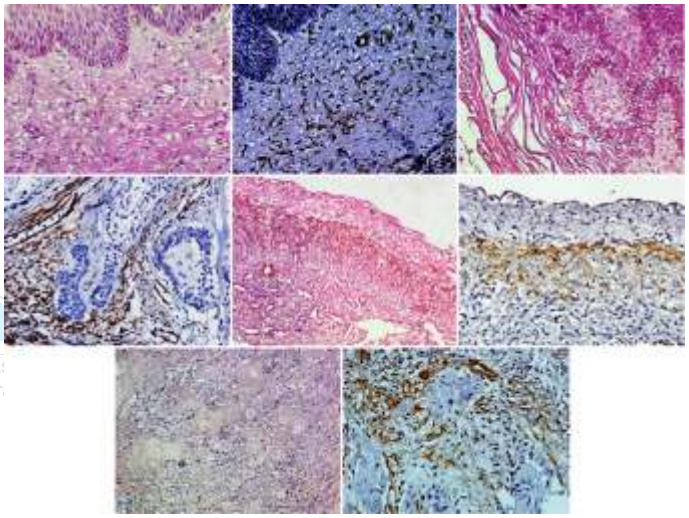

*Fig 5(a) Odontogenic keratocyst (OKC) showing cystic lining epithelium and connective tissue capsule (H&E stain, ×400). (b) Photomicrograph showing a-SMA positive myofibroblasts in the cyst wall of OKC (IHC stain, ×400). (c) Follicular ameloblastoma showing odontogenic epithelial islands in connective tissue stroma (H&E stain, ×400). (d) Photomicrograph showing a-SMA positive myofibroblasts around odontogenic epithelial islands in follicular ameloblastoma (IHC stain, ×400). (e) Luminal variant of unicystic ameloblastoma (H&E stain, ×200). (f) Photomicrograph showing a-SMA positive myofibroblasts in unicystic ameloblastoma (IHC stain, ×400). (g) Malignant epithelial islands in well-differentiated oral squamous cell carcinoma (H&E stain, ×400). (h) Photomicrograph showing a-SMA positive myofibroblasts around tumor islands in oral squamous cell carcinoma (IHC stain, ×400)*
*(Courtesy Soujanya Pinisetti, Ravikanth Manyam, Babburi Suresh, V Aparna: Myofibroblasts in oral lesions: A review. Journal of Oral and Maxillofacial Pathology, 2014 (18), 52- 57*

## Myofibroblasts in Oral submucous fibrosis

Pindborg in 1966 defined Oral submucous fibrosis (OSMF) as an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx.

It is a potential malignant disorder characterised by inflammation and progressive submucous fibrosis. Myofibroblasts are considered to be the key cellular mediator in many fibrotic disorders promoting fibrosis. Studies have been done to identify the presence of myofibroblasts in OSMF and also have revealed that there

is a significant increase in the number of myofibroblasts between early and advanced stages of OSMF. It is also suggested that these cells can also be used as a marker for evaluating the severity of the condition.[18]

## Myofibroblasts in Oral cancer

It is a well known fact that many epithelial tumors are characterised by local accumulation of connective tissue cells and extracellular material. This phenomenon is known as stromal reaction.[19] One of the stromal reaction is the appearance of specialised fibroblasts called Myofibroblasts. Myofibroblasts interact with epithelial cells and other connective cells and may thus control tumor invasion and angiogenesis.[20] Trans-differentiation of fibroblasts into myofibroblasts is a crucial step in tumorigenesis, which is mediated by the growth factors and cytokines secreted by the tumor cells. These myofibroblasts inturn secrete numerous growth factors and inflammatory cytokines that stimulate epithelial cell proliferation. These cells also act along with the immune system and promote angiogenesis, basement membrane degradation, invasion and metastasis.21-23

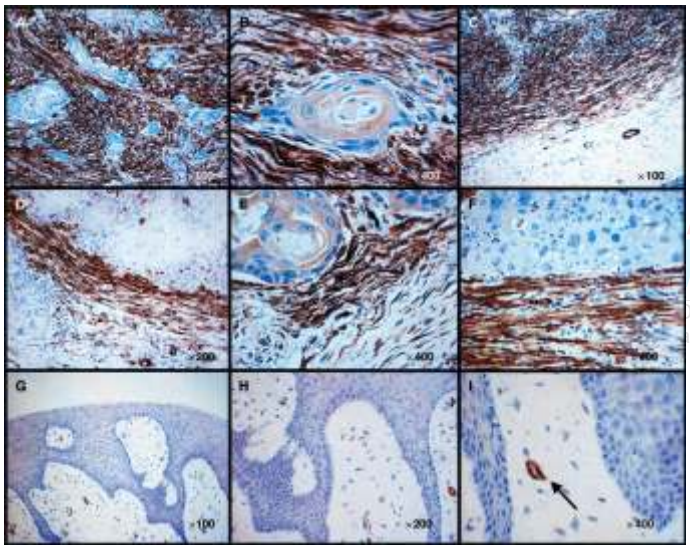

*Fig 6: Stroma of oral SCC contains prominent myofibroblasts in vivo. Immunohistochemistry showing SMA expression by myofibroblasts in oral SCC and lack of expression in benign fibroepithelial hyperplasia. (A) Islands of SCC scattered throughout a myofibroblastic stroma with prominent SMA expression (magnification= x100). (B) A single island of SCC surrounded by SMA-positive myofibroblasts (magnification= x400). (C) Smooth muscle actin expression is concentrated at the tumour margin. Strong SMA expression by myofibroblasts is only detected in the near vicinity of the tumour. Consequently, the margin of the carcinoma appears sharply defined where it abuts 'normal' fibroblastic tissue (magnification= x100). (D–F) Strong induction of SMA expression immediately adjacent to islands of SCC (magnification= x200, x400, x400, respectively). This was usually seen adjacent to the invasive margin at the tumour periphery. (G–I) Lack of SMA expression in fibroblasts of benign fibroepithelial hyperplasia (magnification= x100, x200, x400, respectively). The arrow in (I) indicates positive SMA staining of smooth muscle in the wall of a blood vessel, which serves as an internal positive control.*

*(Courtesy: M P Lewis, K A Lygoe, M L Nystrom, W P Anderson, P M Speight, J F Marshall and G J Thomas. Tumour-derived TGF-β1 modulates myofibroblast differentiation and promotes HGF/SF-dependent invasion of squamous carcinoma cells. British Journal of Cancer (2004) 90, 822-832.*

## Conclusion

Myofibroblasts are ubiquitous cells with similar properties and functions that play significant roles in morphogenesis, organogenesis, and wound healing as well as in disease. As they are present in virtually all tissues, it is possible that they may play a role in multisystem diseases. Understanding the role of the stromal cells and ECM will allow us to identify more precise prognostic markers and potential device new therapeutic options and prevent various diseases caused by these miraculous multipotential cells. Studies can help us to use only beneficial effects of myofibroblasts and control their activation wherever they act hyperactive.

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