



### Myofibroblasts Role in Wound Healing of Eyelid Lesions

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#### Abstract

Activated myofibroblasts play an exert role in wound healing after examining 120 specimens of eyelid lesions, we concluded that an increased number of myofibroblasts was detected in inflamed benign neoplasms and this is possibly related to an improvement in the healing process.

#### Keywords

Myofibroblasts, Wound healing, Eyelid, Inflammatory neoplastic

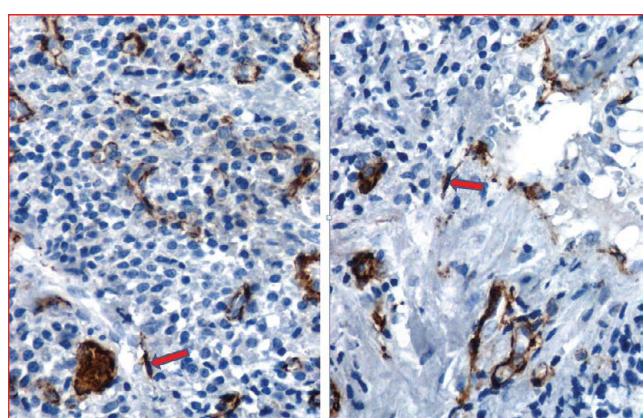
#### Introduction

In injured tissues, the repair of the extracellular matrix is organized by activated myofibroblasts. The latter cells support harmonious mechanical tissue organization [1]. At the end of the normal repair process, myofibroblasts disappear by apoptosis but in pathological situations, myofibroblasts likely remain leading to excessive scarring [2,3].

The eyelids are movable folds of tissue the core of which is represented by a tarsal plate, composed of dense connective tissue, embedded in loose connective tissue. The correlation between the healing process of surgically treated eyelid lesions and histological parameters is worth investigating.

#### Methods

Upon approval by the Bioethics Commission of the School of Medicine of the National and Kapodistrian University of Athens, one hundred and twenty surgical specimens of the eyelids were grouped (Table 1), taking into consideration their inflammatory



**Figure 1:** Detection of  $\alpha$ -SMA-positive myofibroblasts (arrows) among chronic inflammatory cells in the stroma of a papilloma (Immunoperoxidase stain, x400).

**Table 1:** Samples distribution. Each group consisted of thirty specimens.

Group 1 (non neoplastic, non inflammatory)	Group 2 (non neoplastic, inflammatory)	Group 3 (neoplastic, non inflammatory)	Group 4 (neoplastic, inflammatory)
Fibrosis	Fibrosis	Fibrosis	Fibrosis
Minor presence of elastic fibers	Minor presence of elastic fibers	Minor presence of elastic fibers	Increased elastic fibers
Relative absence of myofibroblasts	Relative absence of myofibroblasts	Relative absence of myofibroblasts	Significantly higher numbers of myofibroblasts ( $p = 0.023$ )

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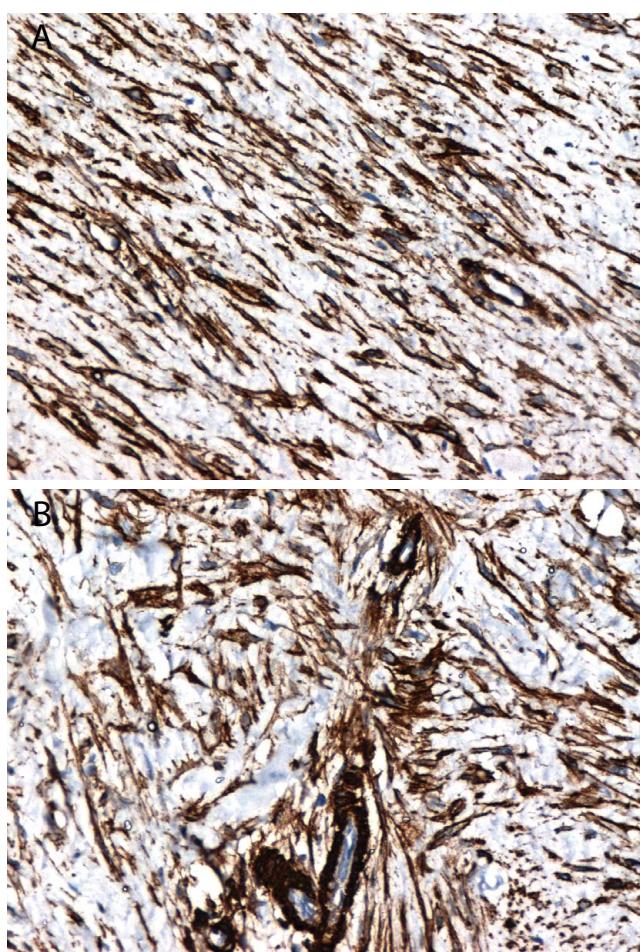
and/or benign neoplastic nature, the presence or absence of fibrosis and elastic fibers (Masson Trichrome and Verhoeff-van Gieson stains) and the quantitative presence of myofibroblasts (after immunostaining for  $\alpha$ -SMA). The number of myofibroblasts was counted among at least 50 high power fields (HPFs, range number of HPFs examined 50-85) by two separate pathologists (G-H.T. and A.C.L.). The quantitative differences among the various groups were investigated by basic statistics at the present stage (student's t-test); the level of statistical significance was set at  $p < 0.05$ . The healing process was clinically evaluated in periods of 1 week, 3 weeks and one month after surgery.

In all cases, we used exactly the same surgical method. The main goal of surgical treatment was to remove the lesion entirely, with the presence of a healthy part of tissue. The anatomical restoration of the eyelid is known to depend on the extent of the total thickness of the horizontal resection. The complete reconstruction of the anterior and posterior part (separated by the brown line) was accomplished according to the size of the deficit.

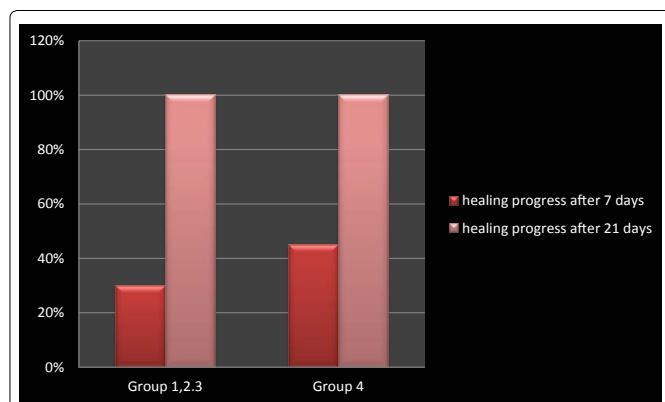
## Results

Myofibroblasts were identified as single  $\alpha$ -SMA-immunopositive spindle shaped cells within inflamed/fibrous tissue (Figure 1).  $\alpha$ -SMA was also expressed in vessel walls and bundles of smooth muscle fibers; of course, this expression could be easily disregarded on morphological grounds (Figure 2).

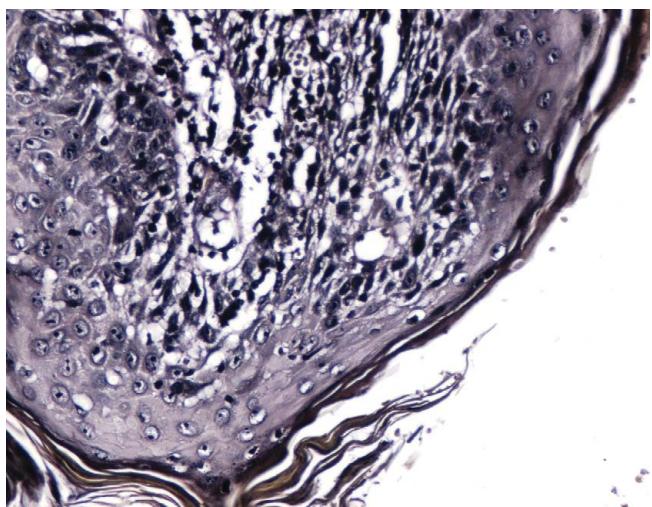
We observed that in inflamed papillomas, myofibroblasts were identified in significantly higher numbers than in the other categories of the examined specimens (Table 1). The presence of inflammation specifically in lesions of neoplastic nature appeared thus to promote the accumulation of myofibroblasts whereas the latter cells tended to be absent in inflamed, non neoplastic lesions and in non inflamed, neoplastic lesions. This finding, among other possible



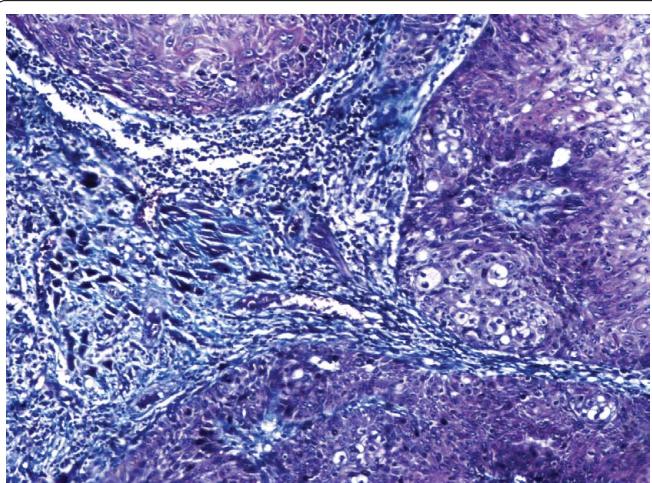
**Figure 2:** Abundant myofibroblasts in the stroma of Group 4 specimens, distinct from  $\alpha$ -SMA-positive cells in vessel walls (Immunoperoxidase stain, x400).



**Figure 3:** Graphics highlighting the rapid healing of Group 4 specimens.



**Figure 4:** Increased amount of elastic fibers in the fibrovascular stalk of an inflamed papilloma (Verhoeff-van Gieson x400).



**Figure 5:** Fibrosis at the base of an inflamed papilloma (Masson stain, x100).

factors, may be related to the former samples' comparatively improved clinical restoration.

In all groups, the induced wounds expanded after surgery to variable extents. By the 3rd day after surgery, all wounds had an area that approximately exceeded  $1.5 \text{ cm}^2$ . In groups 1,2, and 3, the wound area decreased from the 5<sup>th</sup> day with a relatively rapid rate (30% reduction after 7 days and 100% reduction after 21 days) (Figure 3). Group 4 showed the most rapid rate of wound healing (45% reduction after 7 days and 100% reduction after 21 days) (Figure 3). So, by the end of the 21<sup>st</sup> day, these wounds were completely closed in all group 4 samples.

## Discussion

Wound healing involves the integration of complex biological processes of interaction among several types of cells, intercellular matrix and signaling factors [1,2]. Appropriate concordance of numerous aspects, such as blood clotting, inflammatory cell infiltration, cellular proliferation, neoangiogenesis and remodeling of extracellular matrix, enhances epidermal cell proliferation over dermal granulation tissue [3].

The macroscopic evaluation on the wounds of the eyelids in the four groups showed that there were differences in the time it took the crusts to fall off. Healing was slower among the lesions that exhibited a lack of myofibroblasts (groups 1,2,3). Interestingly, in group 4, where the healing process was faster, the presence of myofibroblasts

was accompanied by a considerable amount of elastic fibers (Figure 4 and Figure 5). The presence of myofibroblasts within inflammatory infiltrates might be promoted by tumor-stromal interactions, even in tumors of a benign nature.

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