Stromal myofibroblasts in focal reactive overgrowths of the gingiva

Abstract: Focal reactive overgrowths are among the most common oral mucosal lesions. The gingiva is a significant site affected by these lesions, when triggered by chronic inflammation in response to microorganisms in dental plaque. Myofibroblasts are differentiated fibroblasts that actively participate in diseases characterized by tissue fibrosis. The objective of this study was to evaluate the presence of stromal myofibroblasts in the main focal reactive overgrowths of the gingiva: focal fibrous hyperplasia (FFH), peripheral ossifying fibroma (POF), pyogenic granuloma (PG), and peripheral giant cell granuloma (PGCG). A total of 10 FFHs, 10 POFs, 10 PGs, and 10 PGCGs from archival specimens were evaluated. Samples of gingival mucosa were used as negative controls for stromal myofibroblasts. Oral squamous cell carcinoma samples, in which stromal myofibroblasts have been previously detected, were used as positive controls. Myofibroblasts were identified by immunohistochemical detection of alpha smooth muscle actin (α-sm). Myofibroblast immunostaining was qualitatively classified as negative, scanty, or dense. Differences in the presence of myofibroblasts among FFH, POF, PG, and PGCG were analyzed using the Kruskal-Wallis test. Stromal myofibroblasts were not detected in FFH, POF, PG, or PGCG. Consequently, no differences were observed in the presence of myofibroblasts among FFH, POF, PG, or PGCG (p > 0.05). In conclusion, stromal myofibroblasts were not detected in the focal reactive overgrowths of the gingiva that were evaluated, suggesting that these cells do not play a significant role in their pathogenesis.

Descriptors: Gingival Diseases; Gingival Overgrowth; Myofibroblasts; Immunohistochemistry.

Introduction

Focal reactive overgrowths are among the most common lesions of the oral mucosa. The gingiva is an important area affected by these lesions, primarily triggered by chronic inflammation in response to microorganisms in dental plaque. These lesions are composed of one or more of the following connective tissue components:

- collagen,
- bone,
- endothelial cells, and
- multinucleated giant cells.
The most common focal reactive overgrowths of the gingival connective tissue are:
- focal fibrous hyperplasia (FFH),
- peripheral ossifying fibroma (POF),
- pyogenic granuloma (PG), and
- peripheral giant cell granuloma (PGCG).

FFH, also known as irritation fibroma, is a focal reactive hyperplasia of fibroblasts with overproduction of collagen. POF is a focal reactive hyperplasia of fibrous connective tissue presenting bone formation. PG is a focal reactive growth of granulation tissue with marked proliferation of endothelial cells and blood vessel formation. PGCG is a focal overgrowth composed of mononuclear and multinucleated giant cells.

Myofibroblasts are differentiated fibroblasts that express alpha smooth muscle actin and that have intermediate characteristics of both classic fibroblasts and smooth muscle cells. Transdifferentiation by TGF-β1 stimulation is its most common origin. Myofibroblasts synthesize and degrade extracellular matrix components during inflammation and during the process of tissue repair and remodeling. Therefore, these cells actively participate in diseases characterized by the fibrosis of organs and tissues. Although the presence of myofibroblasts has been reported in hereditary gingival fibromatosis and drug-induced gingival hyperplasia, few studies have evaluated its presence in focal reactive overgrowths of the gingiva.

Therefore, the aim of this study was to evaluate the presence of stromal myofibroblasts in the main focal reactive overgrowths of the gingiva (FFH, POF, PG, and PGCG). Differences in the presence of myofibroblasts among FFH, POF, PG, and PGCG were also analyzed.

### Methodology

#### Tissues and samples

A total of 10 FFHs, 10 PGs, 10 POFs and 10 PGCGs, taken from archival formalin-fixed, paraffin-embedded specimens, were evaluated. This study was approved by the local ethics committee (CAAE - 0161.0.213.000-07).

### Immunohistochemistry

Myofibroblasts were identified by the immunohistochemical detection of alpha smooth muscle actin (α-sma), a marker for myofibroblasts. Four-micrometer sections from the paraffin-embedded samples were used. Tissue sections were dewaxed with xylene, hydrated using graded alcohols, and treated with 0.6% H₂O₂ to eliminate endogenous peroxidase activity. Antigen retrieval was conducted by heating in a 0.01 M citrate buffer (pH 6.0) for 30 minutes. Subsequently, an anti-α-sma monoclonal antibody was used (clone 1A4, diluted 1:100; Dako Corporation, Carpinteria, USA). The LSAB+ kit (Dako Corporation, Carpinteria, USA) was used for the application of the biotinylated link antibody and of peroxidase-labeled streptavidin, according to the manufacturer’s instructions. The reactive products were visualized by immersing the sections for 3 min in 0.03% diaminobenzidine solution, containing 2 mM H₂O₂. The sections were then counterstained with Mayer’s hematoxylin, dehydrated, and mounted.

Normal vessels’ smooth muscle immunoreactivity for α-sma was used as an internal positive control. Samples of oral squamous cell carcinoma, showing numerous and densely arranged stromal myofibroblasts, were used as a positive control (Figure 1F). Samples of gingival mucosa were used as a negative control for myofibroblasts (Figure 1E). The negative control for α-sma immunoreactivity was performed by omission of the primary antibody.

### Scoring of immunostaining results

Alpha smooth muscle actin-positive stromal cells, showing cytoplasmic immunostaining, were considered to be myofibroblasts. Light microscopy was used to evaluate the immunohistochemical reactions. The myofibroblast immunostaining was qualitatively classified as negative (samples in which no stromal myofibroblasts were detected), scanty (samples showing sporadic stromal myofibroblasts), or dense (samples showing numerous and densely arranged stromal myofibroblasts).

### Statistical analysis

The data were analyzed using BioEstat software,
version 5.0 (Optical Digital Technology, Belém, Brazil). Differences in the presence of myofibroblasts among FFH, POF, PG, and PGCG were analyzed using the Kruskal-Wallis test. The level of significance was established at 5%.

**Results**

The results are illustrated in Table 1 and Figure 1.

No stromal myofibroblasts were observed in FFH (Figure 1A), POF (Figure 1B), PG (Figure 1C), or PGCG (Figure 1D). Consequently, no differences were observed in the presence of myofibroblasts among FFH, POF, PG, or PGCG (p > 0.05).

**Discussion**

Myofibroblasts are differentiated fibroblasts that have morphologic and immunophenotypic features similar to those of smooth muscle cells. In addition to alpha smooth muscle actin, myofibroblasts show immunopositivity for vimentin, non-muscle myosin, and fibronectin. These cells show a spindle-cell or stellate-cell morphology, an eosinophilic cytoplasm and an abundant pericellular matrix. Moreover, these cells display the typical ultrastructural features of secreting cells (a prominent rough endoplasmic reticulum and Golgi apparatus producing secretion granules), as well as peripheral myofilaments, fibronexus junctions, and gap junctions.

Myofibroblasts synthesize and secrete cytokines, inflammatory mediators, extracellular matrix proteins, matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases (TIMPs). Due to their ability to secrete and degrade extracellular matrix components, myofibroblasts actively participate in the morphogenesis of tissues or organs, wound healing, fibrosis, and tumor

**Table 1 - Presence of stromal myofibroblasts in focal fibrous hyperplasia (FFH), peripheral ossifying fibroma (POF), pyogenic granuloma (PG), and peripheral giant cell granuloma (PGCG).**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Presence of myofibroblasts</th>
<th>P-value 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFH (n = 10)</td>
<td>10 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>POF (n = 10)</td>
<td>10 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>PG (n = 10)</td>
<td>10 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>PGCG (n = 10)</td>
<td>10 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

1 P-value was obtained using the Kruskal-Wallis test.
Stromal myofibroblasts in focal reactive overgrowths of the gingiva

invasion.\textsuperscript{24,25}

Despite the relevance of myofibroblasts in diseases characterized by fibrosis,\textsuperscript{14,15} few studies have evaluated these cells in gingival overgrowths.\textsuperscript{16-20} In granulation tissue, myofibroblasts undergo apoptosis after wound healing.\textsuperscript{11,13} However, during fibrosis, the continuous presence of TGF-β\textsubscript{1} should inhibit myofibroblast apoptosis,\textsuperscript{26} resulting in their accumulation, mainly in tissues presenting unremitting inflammation.\textsuperscript{11,13} As TGF-β\textsubscript{1} levels are 100-fold greater in gingival inflammatory processes, such as periodontitis,\textsuperscript{27} and as gingival fibroblast transdifferentiation into myofibroblasts, through TGF-β\textsubscript{1} stimulation, has been reported,\textsuperscript{28} the evaluation of myofibroblasts in gingival inflammatory lesions is required. Therefore, this study aimed to evaluate the presence of myofibroblasts in the main focal reactive overgrowths of the gingiva.

FFH is characterized by the hyperplasia of fibroblasts with overproduction of collagen.\textsuperscript{2,5} POF is characterized by hyperplasia of fibrous connective tissue, nevertheless presenting bone formation.\textsuperscript{9} In this study, no myofibroblasts were detected in FFH or POF. These results are in agreement with those of previous reports\textsuperscript{20,21} and suggest that myofibroblasts are not significant in the pathogenesis of FFH or POF, despite their high fibroblast activity. This finding can be explained by the low TGF-β\textsubscript{1} levels in these lesions or by the presence of myofibroblast inhibitors, such as INF-γ, which can inhibit gingival myofibroblast transdifferentiation.\textsuperscript{28}

This is the first study evaluating myofibroblasts in PG, a focal reactive overgrowth with marked proliferation of endothelial cells and blood vessel formation.\textsuperscript{5} Despite its similarity to granulation tissue, an important site of myofibroblasts,\textsuperscript{11,13} these cells were not detected in any of the 10 PG samples that were evaluated.

Although previous reports have detected myofibroblasts in PGCG,\textsuperscript{19,21,22} no myofibroblasts were observed in the 10 PGCG samples evaluated in this study. This divergence is likely a consequence of methodological differences because one study used a histochemical marker for myosin, as well as electron microscopy, to detect myofibroblasts,\textsuperscript{19} and the other used immunohistochemical detection of HHF-35, a muscle-actin-specific antibody.\textsuperscript{21} Nevertheless, another one of the studies\textsuperscript{22} detected myofibroblasts in PGCG using electron microscopy and immunohistochemical detection of alpha smooth muscle actin. An additional hypothesis is that myofibroblasts have been occasionally identified in PGCG because the former report evaluated only 5 samples,\textsuperscript{19} and the second study detected myofibroblasts in just 2 of 10 samples.\textsuperscript{21} In fact, myofibroblasts have also been sporadically detected in central giant cell granuloma,\textsuperscript{29} a lesion histologically similar to PGCG. Moreover, it is possible that myofibroblasts arise as cells in healing processes due to the ulceration of the primary lesions and not as a major player in the pathogenesis of these gingival overgrowths. Finally, it is important to emphasize that PGCG is composed of mononuclear and multinucleated giant cells\textsuperscript{3,4,6} that show immunohistochemical markers of macrophages and osteoclasts.\textsuperscript{30}

Conclusion

Stromal myofibroblasts were not detected in the focal reactive overgrowths of the gingiva that were evaluated, suggesting that these cells do not play a significant role in their pathogenesis.

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References


