Tissues, Pathology, and Diagnostic Microscopy

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Angiogenesis in the reparatory mucosa of the mandibular edentulous ridge is driven by tip cells and influenced by circulating fibrocytes

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Angiogenesis is the process that generates new blood vessels and capillaries from pre-existing blood vessels. This process initially involves proliferation, sprouting, and migration of endothelial cells [1; 2]. The newly generated blood vessel sprout is guided by migrating tip cells [3-7].

It was aimed to perform a qualitative study in order to assess the guidance by tip cells of the endothelial sprouts in the repairing mucosa of the edentulous mandibular crest. In addition, the suitable stromal immune phenotypes for oral mucosa healing were evaluated.

Reparatory mucosa of the edentulous alveolar crest of the mandible was collected prior to healing abutment placement, as well as additional samples of mandibular ridge mucosa collected from patients prior to third molar extractions. The samples were prepared for immunohistochemistry (IHC) and transmission electron microscopy (TEM) examinations. Anti-CD34, anti-CD117(c-kit), anti-PDGFR-α, anti-Mast Cell Tryptase Ab-2, anti-vimentin, anti-CD44, anti-CD45, anti-CD105, anti-α-SMA, anti-FGF2, and anti-Ki67 primary antibodies were used.

In IHC, different stages of sprouts initiation, extension, bridging and lumenization of the newly formed tubes were evidenced in the reparatory samples (Figure 1). By CD34 labeling there were identified extensive processes of sprouting angiogenesis within the lamina propria of non-reparatory samples of oral mucosa. The endothelial stalk cells of resident microvessels were positively stained, as also were the endothelial tip cells (ETCs) involved in active and extensive processes of angiogenesis, in the papillary and the reticular layers. α-SMA positive vascular smooth muscle cells and pericytes were found in both reparatory and control samples. FGF2 was expressed in non-reparatory, but not in reparatory stromal samples. Stromal cells presenting a specific immune phenotype (α-SMA/CD34/CD45 positive) were embedding the microvascular beds, and were identified as being circulating fibrocytes (CFCs). These cells had particular ultrastructural features, different from fibroblasts and immune cells. Morover, ETCs were accurately identified in TEM, penetrating the stromal compartment. Thus, the ETCs were suited for direct interactions with stromal cells. Two patterns of ETCs prolongations were encountered: (a) filopodia, located beneath the endothelial basal lamina, embedded within it, or projected within the stromal compartment; (b) long prolongations emerging from the basal lamina of the endothelial tubes and freely penetrating the stroma. These later processes were moniliform; hook-like collaterals were arising from the dilated segments, suggestive for a staged migration [6].

FGF acts as a key factor in sprouting angiogenesis [8; 9]. The fact that FGF2 was expressed only in non-reparatory samples, suggests that FGF2 acts in physiological reparatory processes, but it may not intervene in wound healing. The pattern proposed is that CFCs contact ETCs, and consequently, when a new endothelial tube is established, the CFCs could be recruited as pericytes [6].

It can be concluded that maintenance and healing of oral mucosa are supported by extensive processes of angiogenesis, guided by ETCs that, in turn, are influenced by the CFCs that populate the stromal compartment both in normal and reparatory states. Therefore, CFCs could be targeted by specific therapies, with pro- or anti-angiogenic purposes.

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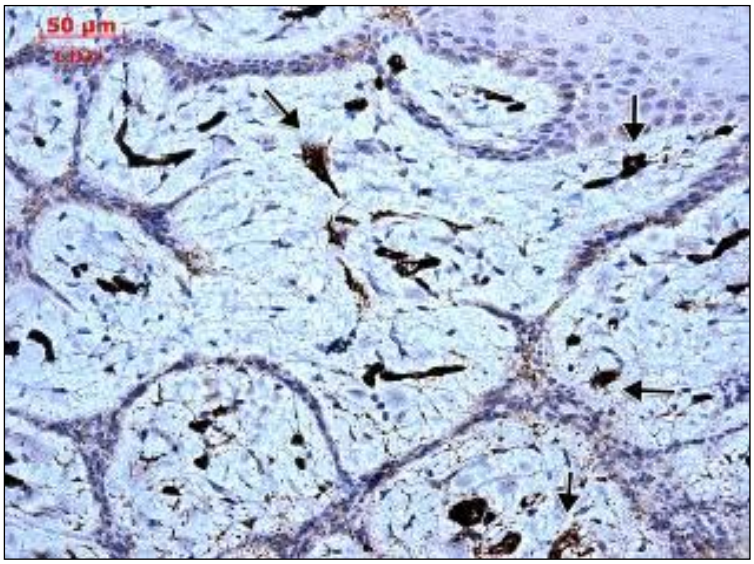

Figure 1. CD34 immune labeling of reparatory oral mucosa. Processes of sprouting angiogenesis are identified (arrows).