The establishment of an efficient soft tissue seal around a dental implant is a prerequisite for implant success. The formation of the tissue barrier is the result of a wound healing process that has been largely documented in animal models (Berglundh et al., 2007). However, data on human oral mucosa are scarce. Recently, we proposed a novel human model to evaluate the morphogenesis of the mucosal attachment to implants (Tomasi et al., 2013). In this study we aimed at characterizing and quantifying tissue adhering to the implant surface by SEM.

Patient informed consent was obtained and after implant installation, a custom-designed experimental abutment was connected to the implant. Soft tissue biopsies and titanium abutment were collected after 12 weeks of tissue healing by the use of a circular cutting device. The whole system was placed in 4% formalin and transferred to a 70% ethanol solution after 48 h. After fixation, the tissue was separated from the experimental abutment using the previously described “fracture technique” (Berglundh et al., 2007).

A total number of 6 experimental abutments were processed for high vacuum SEM to qualitatively and quantitatively reveal biological remnants. Samples were washed twice in phosphate buffer, dehydrated, dried, and gold sputtered. BSE images were acquired at a 15 KeV showing compositional contrast between titanium surface and biologic debris. One set of low magnification (80x) high resolution (2576x1936 pixels) images was acquired per each of the four abutment facets (Figure 1). Each set was digitally processed creating a single high resolution 1.5 mm wide image strip per abutment side. To quantify different morphologies within the biological remnants, a 150x150 µm squared grid was superimposed to the image strips. The observation of each single cross point allowed to quantify the percent of the following micro-morphological features: a) plaque, b) uncovered titanium, c) epithelium-like tissue, d) connective-like tissue (Figure 2).

A minimal amount of plaque was found in the coronal side below the mucosal margin, followed by a uncovered titanium area where no bacteria were present. More apically, single or few layers of nucleated cells formed the epithelium-like tissue. In the deepest area, a thicker connective-like tissue showing fibrous morphology was present. Quantitative results showed a mean (SD) amount of 2%(2%), 52%(27%), 20%(13%), 26%(18%) for plaque, titanium, epithelium-like and connective-like tissue respectively, thus showing a mature healed mucosa in strict contact with the abutment surface (Figure 3). SEM investigation and quantification of tissue adhering to the healing abutment can complement histological findings in describing the healing process of human oral mucosa.

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Fig. 1: Image acquisition and processing for features quantification on the experimental abutment surface. a) Set of images acquired by SEM at 80x magnification covering the whole surface of the abutment facet. b) High resolution strip obtained from image montage. Identification of the mucosal margin and supra and sub mucosa areas.

Fig. 2: Representative images for the four micromorphological features found on abutment surface: a) plaque, b) titanium, c) epithelium-like, d) connective like. SEM, original magnification 250x.

Fig. 3: High magnification images of the abutment surface covered by epithelium-like (a) and connective-like (b) tissue. Close interaction of tissue cells with titanium surface is indicated by white arrows. SEM, original magnification 4000x.