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## Comparative morphological analysis of chronic and acute cigarette smoke effects on human keratinized oral mucosa: ex vivo and in vitro studies

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Human oral mucosa is the first target organ of cigarette smoke, a mixture of highly reactive compounds, well-known causes of cellular molecular alterations. Up to now, morphological studies about smoke effects on this organ have not been carried out. The aim of the present work was the morphological evaluation of intercellular junctions and homeostasis after acute and chronic cigarette smoke exposure. For the acute smoking effects, biopsies of keratinized human oral mucosa (KHOM) obtained from young healthy non-smoking women (n=7) were cultured in a Transwell system previously characterized in our laboratory. A comparative morphological analysis was then performed on oral mucosa biopsies from healthy chronic smoking women of the same age (n=5). All biopsies were obtained after informed consent during the extraction of the wisdom teeth. To simulate the acute smoke exposure, each non-smoking KHOM biopsy was divided in two fragments: one was exposed to direct cigarette smoke thanks to an ad-hoc tool, while the other represented the internal control. After histological processing and paraffin embedding of all experimental groups, the expression of two desmosomal cadherins, desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3), and of a cytoskeletal biomarker for basal keratinocytes, i.e. keratin 14 (K14), was evaluated through indirect immunofluorescence experiments. Dsg1 was distributed as physiologically expected after both chronic and acute smoke exposure and immunoreactivity was detected from the middle spinous layer up to the stratum corneum. Dsg3 immunolabelling in controls and acute smokers was evident in the keratinocytes of basal/lower spinous layers, gradually diminished in upper spinous/granular layers, and disappeared in the stratum corneum. In chronic smoking women, Dsg3 significantly decreased in lower/upper spinous layers, remaining restricted to a limited area of the epithelium. K14 distribution in control group and in chronic smokers was confined to the basal keratinocyte monolayer, but, after acute smoke exposure, it was localized also in correspondence of the lower spinous layer. Taken together, these results suggest that the early “response-to-injury” to an exogenous stimulus such as cigarette smoke involves an alteration in the keratinocyte without any impairment of the epithelial barrier function, as shown by the absence of modifications in the molecular composition of desmosomes. Future studies are needed to correlate our morphological data with the proliferation rate in acute and chronic smokers.