Escape from cell death through autophagy in Human Gingival Fibroblast/Streptococcus mitis co-culture treated with Chitlac n-Ag

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Since ancient times, silver has been extensively used to control infections. Silver based medical products have been proved to be effective in retarding and preventing bacterial infections (Chen et al., 2007). In order to prevent silver nanoparticles aggregation a lactose-modified chitosan was shown to be effective in stabilizing colloidal solutions of silver nanoparticles: “Chitlac-nAg” (Travan et al., 2009). Silver ions and nanoparticle are capable to destroy the bacterial cell wall by reacting with sulfhydryl groups on membrane proteins (Kruszewski et al., 2003). Since the cells are capable of internalizing nanoparticles there is the risk of a massive uptake by eukaryotic cells, which eventually leads to their death through oxidative DNA damage (Li et al. 2013)

In the present work we investigated the effects of Chitlac-nAg on primary human gingival fibroblast (HGFs) co-cultured with Streptococcus mitis in the presence of saliva. HGFs were obtained from fragments of healthy marginal gingival tissue, co-cultured with the clinical strain of S. mitis and treated for 24-48h with Chitlac or Chitlac-nAg. Cytotoxicity evaluated by LDH assay showed an increment in LDH release in co-culture in the presence of Chitlac n-Ag and saliva. Oxidative stress detected by means of Reactive Oxygen Species formation highlighted an early ROS presence in samples with Chitlac-nAg and saliva, but this value was similar to control after 48h; apoptotic and necrotic cells were detected by means of Annexin V/PI showing an increase in cell death in HGFs treated with Ag and saliva after 24h, and returned to basal levels after 48h; the uptake of nanoparticles by cells was determined by optical and electronic microscopy revealing the Ag uptake in vesicles. The presence of lysosomes and autophagosomes was verified by Lysotracker and by LC3 respectively. In vitro results showed that in our co-culture model, which mimics the microenvironment of the oral cavity, chitlac n-Ag does not exert cytotoxic effect towards HGFs that are able to execute a homeostasis mechanism through autophagy promoting cell survival.

References

Keywords
Chitlac n-Ag, co-culture, nanoparticles, toxicity, lysosomes, autophagosomes.