Supplementary Material

Infection of human dental pulp stromal cells by *Streptococcus mutans*: shedding light on bacteria pathogenicity and pulp inflammation

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Materials and Methods

Eukaryotic cells/ Streptococcus mutans interaction and culture: DPSCs and HGFs were seeded in 24-well plates at $10^4$ cells/cm$^2$ and cultured in their corresponding medium. After 72 h of culture, cells were washed with DPBS and cultured, overnight, with antibiotic free culture medium. The next day, cells were washed twice with DPBS and 1 mL of antibiotic free culture medium was added. Cells were exposed to live S. mutans with a multiplicity of infection (MOI) of 30 bacteria : 1 cell. During infection, to determine bacteria content in the supernatant, 30 μL of cell culture supernatants were collected each hour (T0, T1, T2 and T3 h). The rate of viable S. mutans was determined after bacterial count on agar plate.

Supplementary Data

Herein, we sought to investigate if DPSCs exert a direct antibacterial effect against S. mutans. Primary cultured human DPSCs were exposed to live S. mutans. HGFs and cell- and antibiotics-free medium were used as controls. Compared to the inoculum, S. mutans content increased significantly following the first hour of incubation ($p < 0.01$) in controls (Fig. SI-1). Despite a delay in the increase in S. mutans content in contact with DPSCs (i.e. starting 2 h, $p<0.02$), these results showed that DPSCs did not exert a direct antibacterial effect on S. mutans; instead, bacteria seem to have great ability to thrive under DPSCs environmental conditions (LaRock and Nizet 2015).
Supplementary Figures

**Fig. SI-1:** *S. mutans* content in the extracellular environment. During A: dental pulp stromal cells (DPSCs), B: human gingival derived fibroblasts (HGFs) and C: cell free-a-MEM interaction time, suggesting that *S. mutans* established an adaptative behaviour in contact with DPSCs to thrive. Results were normalized to the initial inoculum.

**Fig. SI-2:** Count of viable *S. mutans* after 3 h of contact with dental pulp stromal cells (DPSCs). Tests were performed with or without amoxicillin treatment (Amox-protection assay). *S. mutans* challenged with Cytochalasin D-treated DPSCs indicates that F-actin fibres are required for *S. mutans* internalization by DPSCs (Histograms of mean ± SEM, n = 6, Mann & Whitney test).