

The Effects of Fulvic Acid on Established *Streptococcus mutans* Biofilm Formation and Human Gingival Fibroblast Cells

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Shilajit is a traditional medicine used in Asian countries for centuries to treat numerous health conditions, including bone/cartilage repair and regeneration. Prior research suggests that a major active component of shilajit- fulvic acid- may reduce bacteria in the oral cavity, as in a mouth wash. Because shilajit stimulates connective tissue repair and fulvic acid may inhibit bacteria, the effect of fulvic acid on the caries-forming biofilm bacterium, *Streptococcus mutans*, and on gingival fibroblast cells, which mediate connective tissue in repair/regeneration in periodontal disease, was examined. The goal of this research was to determine whether repeated short-term applications of fulvic acid to *S. mutans* biofilm reduced the amount of established bacteria and to find the concentration of fulvic acid that may inhibit gingival fibroblast cell growth. In the bacterial study, *S. mutans* biofilm was grown, and 8 different dilutions of fulvic acid were applied to the same biofilm groups for 10 minutes each day over a 3-day period. Upon crystal violet staining, the optical density (OD) of the wells was obtained using a spectrophotometer. Higher concentrations of fulvic acid demonstrated stronger inhibition on *S. mutans* biofilm formation. 0.04% repeated applications of fulvic acid resulted in a 2-fold decrease in *S. mutans* biofilm formation, which is not observed with a single application. In the gingival fibroblast cell study, cell toxicity and proliferation were examined utilizing LDH and WST-1 assays, respectively. It was determined that an 0.5% solution of fulvic acid had no effects on cell variability and proliferation. This concentration will be used to examine the effect of fulvic acid on the expression of matrix metalloproteinases (MMPs) from gingival fibroblasts, since the MMPs are involved in tissue degradation and repair. This study demonstrates that fulvic acid has significant antibacterial effects and may be safe for oral use up to a certain concentration.

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