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## The heterogeneous attack of human tooth enamel by a multi-species oral biofilm

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Acid production by biofilms populated with *Streptococcus mutans* and other oral bacteria is commonly implicated in the development of dental caries in humans. Here we report the results of novel experiments designed to measure the extent of sub-biofilm removal of dental enamel using vertical scanning interferometry and other high resolution microscopies. Multi-species biofilms of *S. mutans, Actinomyces odontolyticus, Fusobacterium nucleatum,* and *Veillonella dispar* were grown anaerobically at 37°C for 72 h in artificial saliva containing 0.2% sucrose for 24 hr to 72 hr on human tooth enamel substrates, over which a portion of the polished enamel surface was masked with an inert polymer. Despite complete biofilm coverage of the substrate, the extent of enamel loss at the biofilm-enamel interface was not uniform with respect to biofilm area, and in select samples appeared strongly related to the distance from the mask boundary. The pattern of pitting appears to reflect the flux of acid at the biofilm-enamel interface. Although the observed variations in enamel loss are poorly understood, it is likely that its distribution is a basic feature of biofilm development related to the proximity of a surface discontinuity, in this case the boundary of the polymer mask. This finding has important implications in the development of secondary caries, and raises the possibility that the organization, productivity, and ecology of oral biofilms are influenced by gross discontinuities in substrate topography.