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Standardizing an in-Vitro Biofilm Model to Study Secondary Caries Formation

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Objectives The biggest issue in achieving the longevity of resin-based composite (RBC) dental restorations nowadays is the onset of secondary caries (SC). This study aimed to evaluate the parameters that influence SC formation in an *in vitro* model of cariogenic biofilm challenge.
Methods Four sound human premolars and 10 molars (ethical committee approval obtained) had their roots removed and their pulp chamber filled. Four Class II cavities were made in each tooth having a cervical margin in dentin. Cavities were filled with resin-modified glass ionomer cement (positive control, HVGIC, Equia Forte) or a conventional RBC (negative control, Clearfil Majesty ES-2 Universal). Each material filled two class II cavities in each tooth, one either mesial or distal and one vestibular or palatal. A pink acrylic relining resin was used to obtain two sledges in which teeth were positioned to simulate interproximal contacts between teeth (Figure 1). Sledges were sterilized and stored in artificial saliva for one week. *Streptococcus mutans* monospecies (Sledge #1) or a mixed flora inoculum (artificial oral microcosm, Sledge #2) biofilm formation was obtained on the specimens' surfaces using a bioreactor and a continuous flow (9.5 ml/h) of undefined mucin medium +1 wt.% sucrose at 37 °C for two weeks. Sledges were scanned using microCT (Skyscan 1176, 9µm resolution, 80KV, 300mA); image reconstruction was performed using proprietary software.
Results The microcosm expressed two times deeper demineralization than *S. mutans* and a broth pH of 3.4 vs. 4.3. In the microcosm model, vestibular and palatal cavity margins were much more affected by demineralization than interproximal ones. The resin sledge protected the underlying tooth structures from demineralization. In both models, RBC showed SC development, while HVGIC showed protection against both SC and the loss of minerals in the tissues around it.
Conclusions Standardizing a microbiological model for secondary caries formation and replication in vitro is paramount to a better understanding of this phenomenon.