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Development of an Ex-Vivo Model to Study Dental Carious Lesions

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**Objectives** An entire tooth culture model has been developed to investigate the pulp regeneration potential upon its interaction with pulp capping materials. This study aims at extending this model for studying the interaction of materials with cariogenic bacteria and the target cells.

Methods Third molars extracted for orthodontic reasons were collected under sterile condition. Occlusal cavities were performed and human saliva and S.mutans suspension were applied to the coronal tooth surface to establish a biofilm. A homemade system was employed to immerse the radicular part of the tooth in supplemented MEM medium for a 3-week tooth culture period, preserving dentin-pulp complex activity. Throughout this period, the cavities were incubated alternatively in a nutrition medium (10% sucros) and fasting periodes (1% sucrose) with measurement of pH variations. After 3 weeks, the biofilm was harvested and analyzed using optical microscopy and Gram staining. Demineralization of hard tissues was assessed using Xray radiograph, visual examination and autofluorescence technology (Soprolife<sup>®</sup>). Finally, histological sections were prepared to evaluate the dentin-pulp complex and cariogenic bacteria progression in the dentin using immunofluorescence (DAPI). Results A decrease of pH to 5.5 was observed after 6h. Visual observation and Gram staining confirmed the presence of bacteria and biofilm production. While no demineralization was observed using X-ray, both visual observation and autofluorescence analysis revealed a modification in the enamel and dentin. Finally, histological sections confirmed a hard tissue disorganization and carious progression through the presence of bacteria in the dentin tubules.

**Conclusions** These preliminary results suggest that the entire tooth culture model holds promise in simulating the carious lesion progression and the subsequent dentin-pulp response. By preserving the dentin-pulp activity and producing a biofilm, this model represents a suitable tool in investigating vital pulp therapy under similar clinical conditions.