

## Effect of dentin bonding systems on the expression of matrix metalloproteinases by odontoblasts

N. Lehmann \*, A. Roméas \*, H. Magloire \*, D. Seux \*, F. Bleicher \*

\* INSERM ERI 16 – EA 1892 : Laboratoire du Développement et régénération des tissus dentaires  
UFR d'Odontologie, Rue Guillaume Paradin, 69372 LYON Cedex 08  
Université Claude Bernard, Lyon 1, France.

**INTRODUCTION:** Recent studies have revealed the contribution of host-derived proteinases to the collagen matrix breakdown in the pathogenesis of dentin caries [1-2]. They could have potential implications in dentin bonding. Indeed, the results of these studies suggest that degradation of incompletely infiltrated zones within the hybridized dentin by host-derived dentin matrix metalloproteinases may proceed in the absence of bacterial enzymes [3]. *In situ* collagen degradation within incompletely infiltrated hybrid layers may also adversely affect the remineralization potential of denuded collagen fibrils *in vivo* [4] and *in vitro* [5]. Thus, the objective of this study is to determine the origin of matrix metalloproteinases: dentin bonding systems influence either the expression of metalloproteinases by odontoblasts or only active host-derived metalloproteinases within the dentin matrix.

**MATERIALS & METHODS:** Ten fresh, non-carious, human third molar teeth were extracted from patients 15-18 years old for orthodontic reasons (with their informed consent). Immediately after extraction, they were stored in the culture medium until used. Radiography was performed to visualize the extent of the pulp tissue. An occlusal cavity was prepared on each tooth with a diamond bur (1.6 mm diameter) under water-spray cooling combined with culture medium. The size of these cavities was standardized so that they did not extend over more than half the dentin thickness. Teeth were randomly assigned to two experimental groups (n=5). A dentin bonding system (Xeno III, Dentsply De Trey, Konstanz, Germany) was applied to each cavity of the first group. A flowable resin composite (Ceram X, Dentsply De Trey, Konstanz, Germany) was applied to all bonded specimens and light cured. Both dentin bonding system and composite were used according to manufacturers' instructions. The adhesive system and resin composite were light cured using a previously tested unit (Astralis 5, Ivoclar Vivadent, Saint Jorioz, France). The second group, without a dentin bonding system was used as control. The teeth were carefully sectioned and thick slices were cultured as described previously [6]. The slices were cultured for 7 days. The samples were fixed

in 4% paraformaldehyde-PBS (48 hrs), washed in PBS, demineralized in acetic acid (1N) for 27 days, washed in distilled water, dehydrated in a graded ethanol series, cleared in toluene, and embedded in paraffin. Microtome serial sections (5 µm) were collected. The sections were cleaned of paraffin and treated for immunohistochemical analysis using Vectastain Elite ABC Kits according to the manufacturer's protocol (Vector Labs, Burlingame, California, USA). Two specific mouse monoclonal antibodies were tested: anti-MMP-2 and anti-MMP-9 (R&D Systems, Lille, France).

**RESULTS:** At T0, no differences in terms of staining intensity or distribution patterns were found for MMP-2 and MMP-9 with and without dentin bonding system. The staining was detected in the odontoblast layer and pulp tissue. After 7 days in culture, intense immunoreactivity was observed for MMP-2 and MMP-9 in the odontoblast layer and the pulp tissue respectively in the group with dentin bonding system.

**DISCUSSION & CONCLUSIONS:** The results of this study show that dentin bonding system directly influences the expression of metalloproteinases by odontoblasts. Further *in vitro* studies need to be performed to validate our hypothesis: metalloproteinases synthesized by odontoblasts may get into the hybrid layer through tubules and dentin fluid and then could contribute to damage to incompletely infiltrated collagen in the hybrid layer.

**REFERENCES:** <sup>1</sup>L. Tjäderhane et al (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions, *J Dent Res* **77**:1622-1629. <sup>2</sup>A.J. van Strijp et al (2003) Host-derived proteinases and degradation of dentine collagen *in situ*. *Caries Res* **37**:58-65. <sup>3</sup>M. Ferrari and F.R. Tay (2003) Technique sensitivity in bonding to vital, acid-etched dentin, *Op Dent* **28**:3-8. <sup>4</sup>Y. Mukay and J.M. Ten Cate (2002) Remineralization of advanced root dentin lesions *in vitro*, *Caries Res* **36**:275-280. <sup>5</sup>D.H. Pashley et al (2004) Collagen degradation by host-derived enzymes during aging, *J Dent Res* **83**:216-221. <sup>6</sup>H. Magloire et al (1996) An *in vitro* model of human dental pulp repair, *J Dent Res* **75**:1971-1978.