## Use of dimethyl sulfoxide (DMSO) to optimize adhesive interface: analysis of its effectiveness on the mechanical and biological properties in dentin bonding

Degradation vulnerability of the hybrid layer is strongly related to the presence of water and proteolytic enzymes, which interfere in the longevity of adhesive restorations. Therefore, this work investigated the mechanism of the solvent dimethylsulfoxide (DMSO) in mechanical and biological properties, through the degree of conversion, microtensile bond strength, gelatin and in situ zymography. In this purpose, the work was divided in two parts: the first evaluated the mechanical properties (degree of conversion and microtensile bond strength) and the second tested the biological properties (gelatin and *in situ* zymography). The experimental design of the study involved three factors: dentin bonding systems (DBS) in five levels (Adper Scotchbond Multipurpose [MP], Adper Single Bond 2 [SB], Clearfil SE Bond [CSE], Adper Single Bond Universal [SU] - conventional modes [ER] and self-etching [SE]), dentin pretreatment in two levels (control - water [C], DMSO [D] in different concentrations, depending on the test performed) and time in three levels (initial - 24hours, 6 months and 30 months). In article 1, the degree of conversion of the DBSs associated with different concentrations of DMSO (0%, 1%, 2%, 5% and 10%) was evaluated by the Fourier Transformed Infrared Spectroscopy (FTIR), beyond the bond strength on dentin pretreated passively with 1% DMSO for 60 seconds, by microtensile test, followed by fracture mode analysis. The results showed that DMSO aided to reach the stability of the adhesives after 30 months. For groups MP, SB and SUA, treatment with DMSO increased bond strength values, while for CSE and SUC the values were maintained. In article 2, the comparison of the enzymatic activity of DBSs associated with DMSO was made by gelatin and in situ zymographies. In gelatin zymography, the activity of purified MMP-2 and MMP-9 enzymes pre-incubated with different concentrations of DMSO (0%, 0.5%, 1%, 2%, 5% and 10%) were evaluated in order to verify whether DMSO has a direct effect on the inhibition of proteolytic enzymes. Then, in situ zymography was performed using dentin pretreated passively with 1% DMSO and 2% CHX for 60 seconds. The tooth-resin slices were evaluated in laser confocal scanning microscopy and the images were quantified at Image J software. Gelatin zymography showed no enzyme inhibition, regardless DMSO concentration. For in situ zymography, pretreatments did not affect initial conditions. After 6 months, there was an increase in activity for MP and SB, using both solutions and

only DMSO, respectively. CSE and SU-SE showed a stabilized gelatinolytic pattern independent of the factors. For SU-ER, both pretreatments showed similar lower fluorescence compared to control. The 30-month evaluation indicates the susceptibility to degradation for the etch-and-rinse systems. The results of this study suggest that DMSO may be a promising strategy to improve the adhesive interface over 30 months and that its effect may be mostly related to its interaction with hydrolytic degradation, rather than potential on inhibition oh the enzymes.