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Influence of location and storage time in cohesive strength of dentin

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Abstract

Objective: The purpose of this study was to evaluate the influence of the time of storage in water at the microtensile cohesive strength of occlusal and cervical dentin.

Methods: Thirty sound human molars were used. The occlusal surface was removed under water irrigation. Two slices of dentin (occlusal and cervical) of 1.0 mm of thickness were obtained. Each slice was sectioned in the mesio distal direction to obtain 5 sticks. The sticks were stored in distilled water at 37°C for 24 hours or 6 months according to the groups (occlusal and cervical). The ultimate tensile strength test was performed at a crosshead speed of 1 mm/min. Cohesive strengths were calculated in MPa. The values of cohesive strength were analyzed by two-way ANOVA and Student-Newman-Keuls, at the 5% of level of significance.

Results: No significant interactions among location of dentin, $P=0.59$. Statistical significant difference was observed between cohesive strength of 24 hours to 6 months of storage ($P=0.018$).

Conclusion: Influence of the time of storage in water on the microtensile cohesive strength was observed. The storage of dentin in distilled water for 6 months reduces the values of cohesive strength. However no influence of dentin location was found.

Keywords: Dentin; tensile strength; collagen; dentin-bonding agents

Influência da localização e do tempo de armazenamento na resistência coesiva da dentina

Resumo

Objetivo: O objetivo foi avaliar a influência do tempo de armazenamento em água na resistência coesiva à microtração da dentina oclusal e cervical.

Métodos: Trinta molares humanos hígidos foram utilizados. A face oclusal foi removida sob irrigação constante. Duas fatias de dentina (uma oclusal e uma cervical) com 1,0 mm de espessura foram obtidas. Cada fatia foi seccionada no sentido mesio-distal para obter 5 palitos por fatia. Os palitos foram armazenados em água destilada a 37 °C por 24 horas ou 6 meses, de acordo com o grupo experimental. A resistência coesiva à tração foi avaliada com uma velocidade de 1mm/min e calculada em MPa. Os valores foram analisados por ANOVA de duas vias e Student-Newman-Keuls, considerando um nível de significância de 5%.

Resultados: Não foi encontrada interação estatisticamente significativa entre localização da dentina ($P=0,59$). Foi encontrada diferença significativa entre a resistência coesiva após 24 horas e 6 meses de armazenamento ($P=0,018$)

Conclusão: Foi observada influência no tempo de armazenamento em água na resistência coesiva da dentina. O armazenamento em água destilada por 6 meses reduz a resistência coesiva da dentina. Entretanto não foi encontrada influência da localização na resistência coesiva da dentina.

Palavras-chave: Dentina; resistência à tração; colágeno; adesivos dentinários

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Introduction

Dentin is a mineralized tissue which consists of approximately 50% inorganic material (hydroxyapatite), 30% organic matrix which is composed mainly of type I collagen (85%) and 20% water by volume [1]. Morphologic structure is characterized by tubules with a diameter of approximately 0.9 µm at the dentin-enamel junction, increasing to about 2.5 µm at the pulpal surface [2]. This heterogenic substrate with organic content and moist environment is adverse to hybridization leading to a interface more prone to degradation.

The long term maintenance of bond strength of dentin/adhesive interface is of paramount importance on durability of restorative procedures. Hydrolytic degradation of the interface tooth/resin over time has already been showed in "in vitro" [3] and "in vivo" [4]. Dentin/resin interface degradation is related to the tissue permeability and subsequent deterioration of the hybrid layer [5] and the by the autolytic endogenous metalloproteinases that are activated during acid etching [6].

The development of adhesive system requires methods to evaluate the effectiveness of bond strength to tooth substrate. Tensile bond strength with reduced cross sectional area - "microtensile bond strength" (µTBS) – proposed by Sano [7], in 1994, seems to be a simple and reliable method. Moreover, µTBS presents a moderate correlation with clinical outcomes for adhesive systems [8]. Several aspects could influence the dentin/resin bond strength such as dentin depth and tubule orientation [9]. Few studies that have test to determine the relationship between the structure and the mechanical properties of dentin [10-13], using different methods, found different values of dentin cohesive strength. In addition, some studies [7,14], using etch-and-rinse adhesive system in microtensile tests, have found bond strength values higher than some values measured as cohesive strength of dentin [10,15].

However the behavior of the dentin after a long term of storage in water is still unknown. So the purpose of this study was to evaluate the influence of the time of storage in water on the microtensile cohesive strength of occlusal and cervical dentin.

Methods

Thirty sound human molars were used. The teeth were stored in distilled water at 4°C up to three months before the test. The roots were removed and the pulp chamber was cleared. The occlusal surface was removed under water irrigation. Two slices of dentin (occlusal and cervical) of 0.7 mm of thickness were obtained from each tooth with a Low Speed Saw (Lake Blaff, IL, USA). Each slice of dentin (occlusal and cervical) was sectioned in the mesio distal direction to obtain 5 sticks of approximately 0.5 mm^2 (0.7×0.7mm) of cross sectional area. Caution was taken to verify superficial defects on the dentin surface with a stereoscope at 40× magnification.

The sticks were stored in distilled water at 37°C for 24 hours or 6 months according to the groups. The distilled water was changed every month. The ultimate tensile strength test was performed in a universal test machine Emic DL-2000 (Emic, São José dos Pinhais, Brazil) at a crosshead speed of 1 mm/min. Cohesive strengths were calculated in MPa, dividing the force (N) for the area (mm^2). The values of cohesive strength were analyzed by two-way ANOVA (time and location) and Student-Newman-Keuls, at the 5% of level of significance.

Results

Results from the ultimate tensile strength tests are described in Table 1. Two-way ANOVA revealed no significant interactions among location of dentin (occlusal or cervical) and storage time (P=0.59). Despite the location of dentin (P>0.05), both presented lower ultimate cohesive tensile strength in 6 months than 24 hours (P<0.05).

Table 1. Means and standard deviations for ultimate cohesive tensile strength values, in MPa.

Tooth portion	24 hours	6 months
Occlusal	103.73 (±4.71)[a]	81.99 (±5.22)[b]
Cervical	101.41 (±4.79)[a]	85.12 (±5.58)[b]

Different small letters indicate statistically significant difference in each row (P<0.05).

Discussion

Dentin is a complex tissue composed by organic and inorganic contents and some issues should be considered to use dentin as a substrate for bond strength tests, such as storage time before the test and location of the dentin to perform the adhesion. In the present study the storage time significantly affected the ultimate cohesive strength of dentin. Despite of dentin depth (occlusal or cervical), the cohesive strength of dentin was higher in 24 hours than in 6 months.

Macora and San-Nicolás [16] showed values of cohesive strength of adhesive interface adjacent dentin similar in the present study. However, other authors that tested the cohesive strength of dentin using a shear and tensile test showed lower values [7,10,12,15,17] than the present results. This difference could be explained by the design of the testing methods employed, were the area tested is smaller in the microtensile test than other tests.

The extracted teeth shall be stored in distilled water before to perform adhesive tests [18]. After the restoration procedure, the dentin, with adhesive interface, is storage again in water. To evaluate the collagen degradation as well as, to assess the longevity of the adhesive interface is used aging of the specimens in water for long periods of time. This analysis seems to be related to the clinical longevity of adhesive restorations in class V [8]. However, the maximum storage period and the possible damages to inorganic and organic structure of dentin are still unknown. The influence

of this period at adhesion values is uncertain, because the cohesive strength of dentin could be lower than adhesive bond strength. In this study, 6 months of storage reduces the cohesive strength of dentin. However, the mean values of 6 months seems to be at least similar than adhesive systems with high bond strength values [19].

During the storage time, the teeth were stored in distilled water and this was changed monthly. In other study the significant reductions in bond strength were observed after both long-term storage periods in water or oil [20], probably the water storage increase the degradation of dentinal collagen. Hydrolytic degradation of the interface components, such as dentinal collagen and resin, due to water sorption, could be enhanced by enzymatic degradation [19].

Technical issues involved in bond strength specimen preparation are critical for reliable values of bond strength tests. The depth of dentin was previously related as an important issue [17]. In this study was found no statistical difference between occlusal and cervical dentin. However morphological characteristics could be more important to bond strength values than mechanical properties of dentin. In the present study the failure pattern analysis was not performed. This is a limitation of this study, however the failure pattern analysis to assess only one type of substrate is still uncertain. The similar cohesive strength of dentin in occlusal or cervical indicates that the probability of the mechanical properties influence the bond strength values are not according to the location of the dentin.

Hydrolytic degradation of dentin should be taken into account. The results of this study indicate a reduction in cohesive dentin strength in 6 months. However, the hydrolytic degradation of the polymer and hybrid layer could supplant this degradation, leading an absence of influence of the dentin degradation at the results of bond strength tests after 6 months. The storage of dentin in distilled water for 6 months reduces the values of cohesive strength. However no influence of dentin location was found.

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