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Tatiana Tambara Fröhlich

DENTES DECÍDUOS BOVINOS PODEM SUBSTITUIR OS DENTES DECÍDUOS HUMANOS EM TESTES DE RESISTÊNCIA DE UNIÃO?

Santa Maria, RS
2017

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de concentração em Odontologia, ênfase em Odontopediatria, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do titulo de **Mestre em Ciências Odontológicas**.

Orientadora: Prof. Dra. Rachel de Oliveira Rocha

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Rachel de Oliveira Rocha, Dra. (UFSM)
(Presidente da Banca/Orientadora)

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Jovito Adiel Skupien, Dr. (UNIFRA)

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Alexandre Henrique Susin, DR. (UFSM)

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"Aqueles que passam por nós, não vão sós, não nos deixam sós.
Deixam um pouco de si, levam um pouco de nós." (Antoine de Saint-Exupéry)

RESUMO

DENTES DECÍDUOS BOVINOS PODEM SUBSTITUIR OS DENTES DECÍDUOS HUMANOS EM TESTES DE RESISTÊNCIA DE UNIÃO?

AUTORA: Tatiana Tambara Fröhlich
ORIENTADORA: Rachel de Oliveira Rocha

A avaliação do desempenho de sistemas adesivos em esmalte e dentina de dentes decíduos é justificada pelas diferenças na composição e micromorfologia destes comparadas a de dentes permanentes. A dificuldade de obtenção de dentes decíduos em condições para serem incluídos em estudos laboratoriais faz com que outros substratos sejam utilizados como substitutos. O objetivo desse estudo foi avaliar a resistência de união (RU) de sistemas adesivos ao esmalte e a dentina de dentes decíduos humanos e bovinos, a fim de responder a questão de pesquisa: dentes decíduos bovinos podem substituir os humanos em estudos de adesão? 56 molares deciduos hígidos e 56 incisivos bovinos decíduos foram distribuídos aleatoriamente em oito grupos (n = 7) para cada substrato. Cilindros de resina composta (0,72 mm²) foram construídos sobre os substratos após a aplicação dos sistemas adesivos (Single Bond Universal, estratégias autocondicionante e condicionamento ácido, Clearfil SE Bond e Adper Single Bond Plus) e os espécimes submetidos ao ensaio de microcisalhamento após 24 horas. Análise de Os valores de RU (MPa) foram submetidos a análise de variância de dois fatores e Teste de Tukey (α= 0,05).Um espécime de cada tipo de dente foi preparado para microscopia eletrônica de varredura (MEV) e espectroscopia de raios X por dispersão em energia (EDS). Os valores de RU ao esmalte não foram influenciados signficativamente (p=0,97) pelo tipo de dente. No entanto a interação sistema adesivo vs tipo de dente foi (p=0,04). Em dentina, valores de RU inferiores (p=0.03) foram obtidos para os dentes bovinos. Não foram encontradas diferenças significativamente significantes para a interação sistema adesivo vs tipo de dente (p=0,84). Quando analisado o fator sistema adesivo em esmalte e dentina, independentemente do fator tipo de dente diferença estatisticamente significante foi encontrada em ambos os substratos. Em esmalte o sistema adesivo Single Bond universal na estartégia de condicionamento ácido prévio apresentou o mesmo desempenho do seu controle Single Bond Plus, já a estratégia autocondicionante não foi suficiente para se igualar ao seu controle Clearfil SE Bond. Em dentina os sistemas adesivos apresentaram o mesmo comportamento, com exceção do sistema adesivo Single Bond Plus que obteve os menores valores de resistência de união. Para o sistema adesivo Single Bond Universal a estratégia de uso não influenciou nos resultados em ambos os susbtratos. O esmalte bovino e humano mostrou estrutura semelhante de prismas de esmalte e concentração de Ca e P. Em dentina, uma menor densidade tubular e concentração de Ca e P é obtida nos dentes bovinos decíduos. Diante das diferenças encontradas no substrato dentinário, a substituição de dentes humanos decíduos por bovinos decíduos em estudos de resistência de união só é válida quando o substrato de interesse for o esmalte.

Palavras-chave: Dentina. Dentes decíduos. Esmalte dentário. Resistência de união. Sistemas adesivos.

ABSTRACT

BOVINE PRIMARY TEETH CAN REPLACE HUMAN ONES IN BOND STRENGTH TESTS?

AUTHOR: Tatiana Tambara Fröhlich
ADVISER: Rachel de Oliveira Rocha

The evaluation of the performance of adhesive systems in enamel and dentin of primay teeth is justified by the differences in composition and micromorphology of these compared to permanent teeth. The difficulty of obtaining primary teeth in conditions to be included in laboratory studies causes other substrates to be used as substitutes. The objective of this study was to evaluate the bond strength of adhesive systems to the enamel and dentin of human and bovine primary teeth, in order to answer the research question: can bovine primary teeth replace humans in bond strength studies? 56 sound primary molars and 56 primary bovine incisors were randomly distributed into eight groups (n = 7) for each substrate. Composite resin cylinders (0.72 mm^2) were built on the substrates after application of adhesive systems (Single Bond Universal, self etching and acid etching, Clearfil SE Bond and Adper Single Bond Plus) and the specimens submitted to the 24 hour micro-shear test. The values of bond strength (MPa) were submitted to two-way analysis of variance and Tukey's test ($\alpha = 0.05$). A specimen of each type of tooth was prepared for scanning electron micrograph (SEM) and spectroscopy of Energy dispersive X-rays (ESD). The enamel bond strength values were not significantly influenced ($p = 0.97$) by tooth type. However the adhesive system interaction vs tooth type was ($p = 0.04$). In dentin, lower bond strength values ($p = 0.03$) were obtained for bovine teeth. No significant differences were found for the interaction between adhesive system and tooth type ($p = 0.84$).When analyzed the adhesive system factor in enamel and dentin, regardless of the factor tooth type, statistically significant difference was found in both substrates. In enamel, the Universal Single Bond adhesive system in the previous acid conditioning strategy presented the same performance as its Single Bond Plus control, since the self-etching strategy was not enough to match its Clearfil SE Bond control. In dentin the adhesive systems presented the same behavior, except for the Single Bond Plus adhesive system that obtained the lowest values of bond strength. For the Universal Single Bond adhesive system the use strategy did not influence the results in both substrates. Bovine and human enamel showed a similar structure of enamel prisms and Ca and P concentration. In dentin, a lower tubular density and Ca and P concentration is obtained in primary bovine teeth. In view of the differences found in the dentin substrate, the replacement of primary human teeth by primary bovine teeth in bond strength studies is only valid when the substrate of interest is the enamel.

Key Words: Adhesives. Bond strength. Dentin. Enamel. Primary teeth

SUMÁRIO

1 INTRODUÇÃO

Os dentes decíduos têm papel fundamental no desenvolvimento do sistema estomatognático, sendo importantes para possibilitar o correto estabelecimento da dentição permanente (LÓPEZ-GOMEZ et al., 2016). Assim, um dos principais objetivos da Odontopediatria é a preservação dos dentes decíduos em condições anatomofuncionais, até o período da sua exfoliação (CHAOLLAÍ et al., 2009; CASAGRANDE et al., 2013).

Apesar dos meios e métodos preventivos disponíveis, ainda se faz necessário lançar mão de técnicas restauradoras diante da prevalência de dentes decíduos acometidos por cárie ou traumatismo (PIOVESAN et al., 2011; TELLO et al., 2016). A evolução de materiais restauradores com características adesivas permite realizar restaurações com menor desgaste de tecido dentário, conseguindo-se uma maior preservação da estrutura dentária e também atingir altos padrões estéticos. Os sistemas adesivos associados às resinas compostas são amplamente utilizados na prática clínica e novos materiais são lançados continuamente. Isso faz com que a avaliação do desempenho clínico destes materiais seja necessária. Ainda que estudos clínicos longitudinais sejam ideias para se avaliar a efetividade dos sistemas adesivos, testes laboratoriais são considerados importantes por avaliar, em um menor tempo, a eficiência da adesão ao substrato dentário, auxiliando na previsibilidade do seu desempenho clínico (VAN MEERBEEK et al., 2003, 2010; BAYANE, 2012).

Muitas das informações obtidas a partir dos resultados de estudos laboratoriais em dentes permanentes são extrapoladas para dentes decíduos, apesar das diferenças na composição e morfologia entre eles (OLIVEIRA et al., 2010; LENZI et al., 2013). Nör et al. (1996;1997) mostraram que a dentina de dentes decíduos é mais sensível ao condicionamento ácido, resultando em interfaces adesivas mais espessas comparadas aquelas formadas em dentina de dentes permanentes. Além disso, estudos que compararam a resistência de união à dentina decídua e permanente observaram valores diferentes de resistência de união entre elas (UEKUSA et al., 2006; SENAWONGSE et al., 2004; LENZI et al., 2012; SOARES et al., 2017). Na comparação da resistência de união ao esmalte decíduo e permanente, alguns estudos apresentam resultados semelhantes para os dois substratos (SHIMADA et al., 2002; HOSOYA et al., 2000), já outros apresentam resultados inferiores para o esmalte decíduo quando comparado ao de dentes permanentes (LENZI et al., 2013; PEUTZFELDT; NIELSEN 2004; TEDESCO et al., 2014).

Visto que podem existir diferenças na resistência de união aos substratos decíduos e permanentes, faz se importante que os resultados obtidos em testes realizados em dentes

permanentes não sejam extrapolados diretamente. Porém, há a dificuldade em se conseguir dentes hígidos em quantidades suficientes para serem testados (NAKAMICHI et al., 1983). Essa dificuldade se torna ainda maior quando dentes decíduos hígidos são necessários, pois a sua obtenção depende da doação voluntária da criança e responsáveis quando da sua exfoliação. Além disso, a principal razão para indicação de extração desses dentes é o comprometimento por cárie dentária (BANI et al., 2015; MANSOUR; BAGESUND 2010; AK et al., 2005). Nesses casos os dentes já não estão em condições ideais para serem testados nos ensaios de resistência de união.

Dessa maneira, há a procura por substitutos aos dentes humanos para uso em estudos *in vitro*. As vantagens de usar dentes bovinos estão relacionadas a maior facilidade de serem obtidos em grande número, geralmente livre de defeitos e lesões de cárie e, pelos animais serem sacrificados aproximadamente na mesma idade, conseguir controlar a faixa etária dos dentes e seu tempo de armazenamento (CAMARGO et al., 2008; TANAKA et al., 2008; SFONDRINI et al., 2011). Além disso, eliminam a necessidade de submissão do protocolo da pesquisa para avaliação por um Comitê de Ética em Pesquisa, dado que são provenientes de animais já abatidos (TANAKA et al., 2008).

Diversos estudos têm considerado o uso de dentes bovinos em avaliações de resistência de união de sistemas adesivos (BRONZATO et al., 2016; FRATTES et al., 2017; FORGERINI et al., 2017; AYAR et al., 2017) , seja pela semelhança na composição química comparados aos dentes humanos (TERUEL et al., 2015) ou pelos valores similares encontrados na comparação entre os substratos de diferentes origens (REIS et al., 2004; MUENCH et al., 2000; SAUNDERS et al., 1988). O uso de dentes bovinos em estudos de resistência de união é suportado por recente revisão sistemática, na qual não foram encontradas diferenças significantes entre dentes humanos e bovinos, seja em esmalte ou dentina (SOARES et al., 2016). É válido ressaltar, no entanto, que apenas um (KRIFKA et al., 2008) dos estudos incluídos nesta revisão sistemática, utilizou dentes bovinos decíduos como substrato e ainda que, este não foi considerado na meta-análise por não apresentar os valores de resistência de união segundos os critérios estabelecidos (média e desvio-padrão).

Não obstante, há poucos estudos que validaram o uso de dentes bovinos como substitutos aos dentes humanos decíduos e, assim como na comparação entre dentes permanentes e decíduos humanos (TEDESCO et al., 2014; OZMEN et al., 2015; NICOLOSO et al., 2016; SOARES et al., 2017) , pode-se esperar resultados conflitantes também para esta comparação em dentes bovinos (OESTERLE et al., 1998; SFONDRINI et al., 2011). Estudos laboratoriais avaliaram a possibilidade dos dentes bovinos decíduos serem substitutos

dos humanos em experimentos de abrasão e erosão e constataram, no entanto, diferenças estatisticamente significantes na perda mineral do esmalte e da dentina entre os tipos de dentes (ATTIN et al., 2007; WEGEHAUPT et al., 2008).

Os valores de resistência de união de um sistema adesivo aplicado ao esmalte de dentes bovinos decíduos foram similares àqueles encontrados no esmalte de dentes humanos decíduos, mas o mesmo não foi verificado para o substrato dentinário (TITLEY et al., 2006). Em contrapartida, os dentes bovinos decíduos apresentaram valores similares aos humanos, resultado válido tanto para esmalte quanto para dentina (KRIFKA et al., 2008).Estes estudos, no entanto, apresentam limitações importantes, relativas ao tipo de dente humano utilizado (não informado) e ao ensaio mecânico empregado – cisalhamento, que apresenta como característica intrínseca, o alto percentual de falhas coesivas, cujos valores foram incluídos no cálculo das médias de resistência de união.

Diante dos poucos estudos disponíveis sobre o assunto, as limitações dos estudos já existentes e os resultados divergentes, justifica-se a realização de outros estudos com o objetivo comprovar se o dente decíduo humano pode ser substituído pelo dente decíduo bovino em testes de resistência de união.

2 ARTIGO - ASSESSMENT OF THE INFLUENCE OF BOVINE AND HUMAN PRIMARY TEETH ON THE BOND STRENGTH OF ADHESIVE SYSTEMS

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Assessment of the influence of bovine and human primary teeth on the bond strength of adhesive systems

Tatiana Tambara Fröhlich
Graduate student
Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 97015-270, Santa Maria, RS, Brazil
Contribution to the paper: Consulted on idea, hypotheses, performed the experiments, wrote the manuscript

Tathiane Larissa Lenzi

Professor
Graduate Program in Dental Science, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 97015-270, Santa Maria, RS, Brazil
Contribution to the paper: Contribution substantially to discussion, proofread the manuscript.

Fabio Zovico Maxnuck Soares

Adjunct Professor
Department of Restorative Dentistry, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 97015-270, Santa Maria, RS, Brazil
Contribution to the paper: Contribution substantially to discussion, proofread the manuscript.

Rachel de Oliveira Rocha

Associate Professor
Department of Stomatology, Federal University of Santa Maria, Brazil
Rua Marechal Floriano Peixoto, 1184, 97015-270, Santa Maria, RS, Brazil
Contribution to the paper: Idea, study design, contribution substantially to discussion, proofread the manuscript.

Assessment of the influence of bovine and human primary teeth on the bond strength of adhesive systems

Abstract:

Purpose: To compare the influence of the type of the teeth on the bond strengths of three representative adhesive systems (one etch-and-rinse, one self-etch and one universal system)
Materials and Methods: Fifty-six sound deciduous molars and fifty-six deciduous bovine incisors were used. For each substrate (enamel and dentin), the teeth were randomly assigned to eight groups (n =7) according to the origin of the tooth (human or bovine) and adhesive system (Scotchbond Universal Adhesive, in self-etching and etch-and-rinse modes; Adper Single Bond Plus (control); and Clearfil SE Bond (control)). The adhesive systems were applied to the flat surfaces of the enamel or the dentin and subsequently composite resin cylinders were built (0.72mm²). After 24h microshear bond strength was performed. Bond strength (MPa) were analyzed using two-way ANOVA and Tukey´s post-hoc tests ($\alpha = 0.05$).
Results: The type of teeth did not affected significantly the bond strength of adhesive systems to enamel ($p = 0.97$). The bond strength to dentin of primary bovine teeth has significantly lower than human teeth ($p = 0.03$).
Conclusions: Only bovine deciduous teeth enamel may be considered as a suitable alternative to human deciduous teeth in bond strength tests.

Key Words: Adhesives. Bond strength. Dentin. Enamel. Primary teeth

Introduction

The development of adhesive systems requires laboratory studies to provide information about performance and possible influencing variables. Almost all *in vitro* data on adhesive systems are obtained from permanent teeth and extrapolated to primary ones teeth, even though differences in chemical composition and morphological characteristics [6,19] may explain the different performance of adhesive systems applied to these substrates. [3,14,20,21,31,38,50] Therefore, ideally, laboratorial evaluations of adhesive systems should be done in both primary and permanent teeth.

The use of primary human teeth in *in vitro* studies is being trouble due to ethical limitations, voluntary donation by children and parents or guardians and, mainly due to the difficulty in obtaining sound primary teeth. Severe impairment by caries lesion is the main reason for primary teeth extraction [2, 25], so most of extracted primary teeth are not suitable to be used in bonding studies.

Bovine teeth have been suggested as substitutes for human teeth for several reasons: great similarity in the chemical composition [47], easiness in obtaining in large number, standardization of animal age, diet, generally free of defects and caries lesions and, the same post-extraction time [5, 39, 43]. In addition, the use of bovine teeth dispense the consent and approval by the ethics Committee, since they come from animals already slaughtered.[43]

According to a systematic review, the use of bovine teeth in bond strength studies produces similar results compared to human ones, both to enamel and dentin substrates.[42] However, all of the included studies compared permanent human and bovine substrates, thus, the results must not be used to predict the similarity between primary human and bovine teeth.

Only two previous studies [16, 49] compared the bond strength of adhesive systems to primary bovine and human teeth but their results do not permit to conclude if bovine teeth can

also be used as a substitute of human teeth in primary dentition, since there was no agreement between the findings of the two studies. Besides that, the type of human primary teeth used was not informed and also, cohesive failures, a common finding in shear tests, were included in bond strength values, resulting in an overestimation of real values. So, to the extent of the author's knowledge, the evidences supporting the use of bovine primary teeth as a substitute to human ones on bond strength studies are scarce and unclear. Therefore, the aim of this study was to compare the influence of the type of the teeth on the bond strengths of three representative adhesive systems (one etch-and-rinse, one self-etch and one universal system). The null hypothesis tested was that the bond strengths of the tested adhesive systems to enamel and dentin are similar between bovine and human primary teeth.

Materials and Methods

Specimen preparation

The Institutional Ethics Committee previously reviewed and approved the study protocol. Fifty-six sound human primary molars were selected from a pool of stored human teeth (distilled water at 4º C). Fifty-six freshly extracted bovine primary incisors were selected. All teeth were disinfected in 0.5% chloramine-T solution previously the use.

The sample size was estimated based on previous studies [1,46], considering the coefficient of variation of 20%, power of 80% and level of significance of 5%, with this a minimum of 7 teeth per group was required. Teeth were randomly allocated into 16 groups according to: a) origin of teeth (human or bovine); b) substrate (enamel or dentin); c) adhesive system.

After sectioning the roots in a cutting machine with a low-speed water-cooled diamond saw (Labcut 1010, Extec Co.; Enfield, CT, USA) bovine teeth crowns were

individually embedded in self-curing acrylic resin inside PVC rings (JET Classico; Sao Paulo, SP, Brazil), leaving the labial surfaces exposed. Flat enamel and dentin surfaces were exposed using #600-grit SiC papers under running water. Exposed enamel and dentin surfaces were then washed and dried with oil-free compressed air.

The crows of 28 primary human molars were sectioned mesio-distally, resulting in two sections that were individually embedded in self-curing acrylic resin inside PVC rings (JET Classico; Sao Paulo, SP, Brazil), leaving the labial or lingual surfaces exposed. Flat enamel surfaces were obtained as described to bovine teeth. The remaining primary molars were embedded by the cervical portion in self-curing acrylic resin inside PVC rings and flat dentin surfaces were exposed after removal of the occlusal enamel using a slow-speed diamond saw under water cooling. Dentin surfaces were ground with #600-grit SiC paper under running water.

Bonding procedures

Three representative adhesive systems, a two-step etch-and-rinse (Adper Single Bond Plus), a two-step self-etch (Clearfil SE Bond) and a universal system (Scotchbond Universal Adhesive), were applied to enamel and dentin surfaces according to their respective manufacturers' instructions (Table 1) by a single trained operator. Prior the light curing of the adhesives, starch tubes [45] (0,96mm internal diameter x 1mm height) were carefully positioned, three and four starch tubes were placed over the bonded enamel and dentin surfaces, respectively. After light curing (Emitter C, Schuster; Santa Maria, RS, Brazil), the tubes were completely filled with resin composite (Filtek Z250, 3M ESPE; St Paul, MN, USA) and light cured for 20s. This procedure results in cylindrical composite resin specimens with 0.72 mm^2 of bonded area. After the storage in distilled water at 37º C for 24 h, the starch tubes were easily removed using air/water spray.

Microshear Bond Strength Test (μSBS)

The composite resin cylinders were examined under a stereomicroscope at 10X magnification (Stereo Discovery V20, Carl Zeiss, Rio de Janeiro, RJ, Brazil) and those interfacial gaps, air bubbles or other defects were discarded and replaced. The specimens were attached to a universal testing machine (EMIC DL 1000, São José dos Pinhais, PR, Brazil) and a shear force was applied at a crosshead speed of 1.0 mm/min until failure using a stainless-steel wire loop (0.2 mm in diameter) placed as close as possible to the adhesive interface. A single experienced blind operator performed the tests.

Failure Analysis

All specimens were observed at a stereomicroscope at a magnification of 40x. Failures were classified as interfacial (failure at the resin- dentin interface or mixed with cohesive failure of the neighboring substrates) or cohesive (failure exclusively within the substrate – enamel or dentin; or within the composite resin).

SEM investigation and Energy Dispersive X-Ray Spectroscopy

A section of dentin and enamel of human and bovine teeth was prepared for evaluation in a scanning electron microscope (SEM). Enamel and dentin were etched with 35% phosphoric acid for 90 s and washed with air-water spray for 30 s.[8] The specimens were dehydrated in ascending concentrations of ethanol (50%, 75%, and 95% for 5 min each, and 100% for 3 h) and kept in vacuum for 24h.[27] Subsequently they were gold sputtered an analyzed in a SEM (VEGA3, TESCAN) operated in secondary electron mode with 5.0 kV voltage. Two scanning electron micrographs from each specimens were obtained at a magnification of 500x and 2000x. In addition, energy dispersive X-ray spectroscopy was

performed to determine the chemical composition by element of enamel and dentin for each tooth type.

Statistical Analysis

The experimental unit was the tooth. Thus, a mean of all values obtained from each tooth were considered in the statistical analysis. Statistical analyzes were performed separately for each substrate, enamel and dentin. A normal distribution was confirmed by the Kolmogorov-Smirnov test. The μSBS values were subjected to a two-way Analysis of Variance (ANOVA), considering the adhesive systems and origin of teeth as factors. A post-hoc Tukey test was used to compare means. Statistical analysis was performed using Minitab software (Minitab; State College, PA, USA) at a significance level of 5%. Pre-test failures were assigned the value 0 MPa and were included in the statistical analysis.

Results

The μSBS means, standard deviations, number of tested specimens and premature failures of enamel and dentin human and bovine teeth are presented in Table 2. The origin of teeth (bovine or human) did not affected significantly the bond strength of adhesive systems to enamel ($p = 0.97$). However, the interaction adhesive system *vs* origin of teeth was significant ($p = 0.04$), thus the ranking of the adhesive systems was not identical between bovine and human enamel substrates. Conversely, the origin of teeth significantly affected the bond strength to dentin ($p = 0.03$); higher μSBS values were found to human compared to bovine dentin. No significant differences were found for the interaction adhesive systems *vs* origin of teeth ($p = 0.84$) to dentin substrates. On the other hand, analysis of variance showed that the adhesive system factor was statistically significant ($p=0.00$) in dentin substrate, regardless the origin of teeth. The two-step etch-and-rinse Adper Single Bond Plus presented

the lower bond strength values compared to the other systems, which were similar to each other.

No cohesive failures were found; the failures of all specimens were classified as interfacial failures (adhesive or mixed).

Representative micrographs and EDS analysis of a representative area of human and bovine enamel and dentin are illustrated in Figures 1 and 2, respectively. High magnification of SEM micrographs of enamel substrate revealed the similar structure of enamel prisms and the Ca and P concentration. On dentin micrographs, higher tubular density in human teeth is evident as lower Ca and P concentration in bovine teeth.

Discussion

This study investigated the influence of the origin of the teeth (i.e., primary bovine and human teeth) on the bond strength of three adhesive systems currently used and representative of different bonding concepts. A previous systematic review [42], showed that bovine teeth can be considered as a reliable substitute to human ones on bond strength studies, a finding which was not confirmed in the present study.

To enamel, no differences in bond strength values were found between primary bovine and human teeth, as demonstrated in earlier studies in permanent and primary teeth .[11,16,23,32,49] On the other hand, higher bond strength values were found on primary human dentin. This finding does not seem to be consistent with the previous studies.[11,15,16,24,32,34,35] However, it should be noticed that in these studies, except one [16] , permanent human and bovine dentin were compared. Thus, it might be assumed that the chemical and morphological differences between human primary and permanent teeth [12] that may influence on the performance adhesive systems should also exist in bovine dentition and can explain this result.

Additionally, as suggested previously by Schilke and others [37] ,differences in bond strength values may be related to structural differences and chemical composition of dentin according the tooth origin. In the present study, a higher concentration of tubules could be clearly seen in human primary dentin compared to bovine primary dentin. Likewise, this difference in morphological pattern was also found to human permanent dentin [22] and could be related to different primary dentin depths used; superficial dentin in bovine teeth and deeper dentin in the human molars. The higher bond strength found in the present study to human primary teeth can be a consequence of better resin monomer infiltration in human deeper dentin than in superficial bovine dentin.[28,44,52] Although the bonding performance at different dentin depths is still controversial, especially due to the relation between tubular density and intrinsic moisture, the use of hydrophilic adhesive systems seems to make the bonding to deep dentin less sensible to dentin water content.[30] Besides that, studies have shown that Scotchbond Universal adhesive system it is not so sensitive to the degree of dentine moisture, since it provides similar immediate bond strength to dentin under moist or dry conditions.[17,18]

Moreover, it can be assumed that the lowest calcium and phosphorus concentrations (approximately ten times lower) in bovine primary dentin also explain the lower bond strength values found to this substrate, probably due to the greater diffusion of the inorganic conditioners and the compromised buffering action of the mineral phase of less mineralized dentin.[53] This difference in chemical composition was not found when permanent human and bovine dentin were compared.[10, 47]

Although the evaluation of the influence of the origin of the teeth is the main objective of the study, it is worth discussing the performance of the adhesive systems in each of the considered substrates considered. The ranking of adhesive systems applied to enamel was different according to the origin of the teeth. In human enamel, Clearfil SE Bond presented

higher bond strength values than Scotchbond Universal Adhesive in self-etch mode, which is in agreement with previous studies that point out that multimode adhesive systems showed lower bonding values compared to two-step self-etch systems and that enamel phosphoric acid etching must be done before the prior.[33,36,51] In bovine teeth, significant differences were found between Scotchbond Universal Adhesive in etch-and-rinse strategy and Adper Single Bond Plus, although they are of the same strategy. These differences in the ranking of the adhesive systems according to the origin of the teeth could be explained by the exposed enamel surface in human molars and bovine incisor, resulting in different ways in which the prisms were cut and exposed.[40]

The worst performance of Adper Single Bond Plus in dentin, regardless the origin of the teeth, was also found in previous studies [4,48] and the entrapment of remaining solvent is pointed as the reason for it.[4,9] In bovine teeth, dentin was exposed in longitudinal axial direction, while in human teeth, in a transverse direction, resulting in different tubule orientations, which certainly influence the bonding performance of different adhesive systems.[26] Besides that, shear testing may be affected not only by both dentinal tubule orientation and exposed enamel prisms; but also by the possible morphological differences between human molars and bovine incisors.[13,29] Even so, shear bond test is a simple test procedure and can make clear specific factors that influence the bonding effectiveness [7], such as the substrate related to the origin of the teeth.

Although the search for human teeth substitutes is not new, considering their use in laboratorial studies depends on their obtaining in sufficient number and the ethical limitations, the subject is not yet concluded. While permanent human teeth can be replaced by bovine ones [42], the same does not seem to be valid for primary teeth, according to the results of the present study. The null hypothesis of the study was rejected to dentin but not to enamel, so bovine primary teeth can only reliably replace human teeth in adhesion studies when enamel

is the focused substrate. Further studies are need to validate these findings or to support the use of bovine primary teeth as substitutes to human primary teeth in laboratorial evaluations of adhesive systems.

Conclusion

The origin of the teeth – primary human or bovine influence differently the bond strengths of three representative adhesive systems (one etch-and-rinse, one self-etch and one universal system) to enamel and dentin. While similar values were found to enamel, bovine primary dentin yield lower bond strength values.

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Tables and illustrations

Table 1: Adhesive systems (manufacturers and batch number), composition and application mode *:

Adhesive System/ Batch Number	Main components	Mode of application	
		Self-etching strategy	Etch-and-rinse strategy
Adper Single Bond Plus (3M ESPE, St. Paul, NN, USA) (N677700)	Etchant: phosphoric acid 37% Bond: bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(itaconic) acids, 10% 5-nm-diameter spherical silica particles pH=4,7	N.A.	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Blot excess water 4. Apply 2 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air dry for 5 s 6. Light cure for 10 s
Clearfil SE Bond (Kuraray Dental Inc.,Okayama Japan) (PRIMER: 9N0168 BOND: 9U0269)	Primer: MDP, HEMA, hydrophilic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, water Bond: MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica pH=2,0	Apply primer on dry dentin surface and leave undisturbed for 20 s. Dry with air stream for 5 s to evaporate the volatile ingredients. Apply bond and gently air dry. Light cure for 10 s.	N.A.
Scotchbond Universal Adhesive (3M ESPE, St. Paul, NN, USA) (589525)	Etchant: 37% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane pH=2,7	Actively apply the adhesive to the entire surface for 20 s. Direct a gentle air stream over the adhesive for 5 s or until it no longer moves and the solvent completely evaporates. Light cure for 10 s.	1. Apply etchant for 15 s. 2. Rinse thoroughly. 3. Blot excess water 4. Apply adhesive as for the self-etching mode.

* According to manufacturers' information. Abbreviations: bis-GMA: bisphenol A glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; N.A.: not applicable.

Table 2. Means of μSBS of adhesive systems applied to enamel and dentin of human and bovine primary teeth.[*]

	Enamel		Dentin	
Material	**Human**	**Bovine**	**Human**	**Bovine**
Clearfil SE Bond	19.3 (4.1) [21/1] [A]	16.5 (3.4) [17/0] [A,B]	13.1 (2.3) [28/1]	11.6 (2.6) [27/0]
Adper Single Bond Plus	14.4 (4.0) [22/0] [A,B]	12.2 (3.1) [18/2] [B]	7.12 (3.3) [27/5]	5.8 (1.5) [24/6]
Scotchbond Universal Adhesive (self-etch strategy)	11.7 (3.4) [21/1] [B]	13.9 (3.9) [20/3] [A,B]	12.0 (4.0) [24/1]	9.3 (5.0) [27/6]
Scotchbond Universal Adhesive (etch-and-rinse strategy)	15.3 (4.0) [21/1] [A,B]	19.4 (3.8) [16/0] [A]	13.0 (5.8) [26/5]	9.4 (3.1) [23/3]

* Means (standard deviation) [tested specimens/premature failures]
Identical capital letters indicate the absence of any statistically significant differences to the interaction origin of the teeth and adhesive system (p=0.00).

Figure 1. Representative scanning electron microscopy images and EDS analysis of deciduous human enamel (A) and deciduous bovine enamel (B) .Left-hand images: 500X magnification; right-hand images: 2.000X.

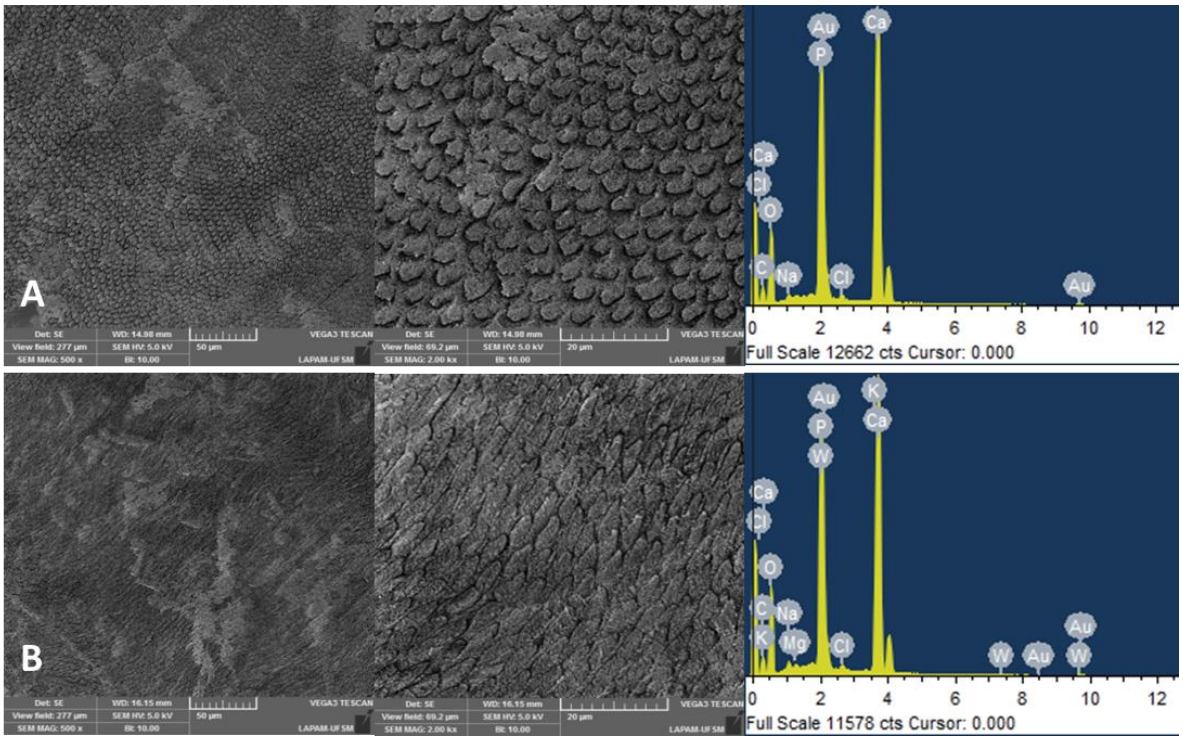
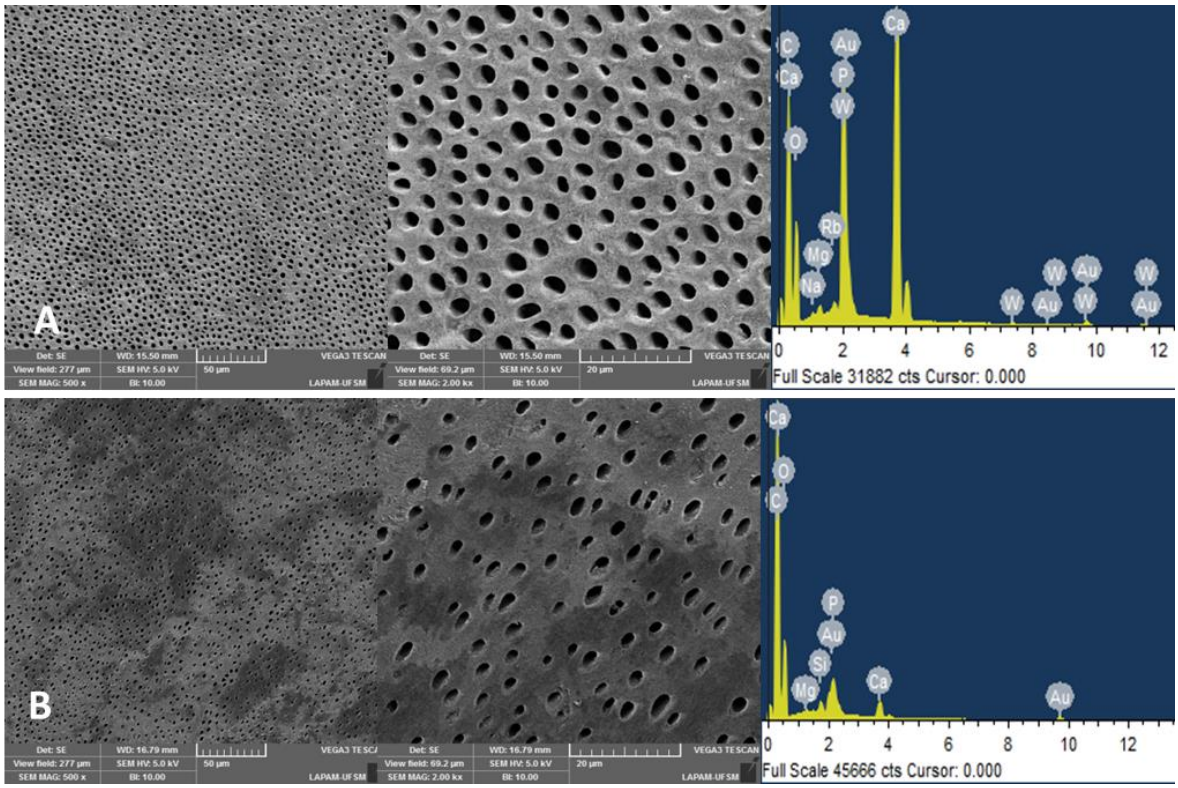


Fig 2. Representative scanning electron microscopy images and EDS analysis of deciduous human dentin (A) and deciduous bovine dentin (B) .Left-hand images: 500X magnification; right-hand images: 2.000X.



3 CONCLUSÃO

Esta dissertação avaliou a resistência de união de sistemas adesivos ao esmalte e à dentina de dentes bovinos e humanos decíduos. Não foi encontrada diferença estatisticamente significante quando avaliado o substrato esmalte, porém em dentina valores significantemente menores foram obtidos para os dentes decíduos bovinos.

Dessa forma, os dentes bovinos decíduos podem substituir os dentes humanos decíduos em ensaios de resistência de união somente quando o substrato de interesse for o esmalte.

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ANEXO

ANEXO A- CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DA UFSM

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Dentes bovinos decíduos podem substituir os dentes decíduos humanos em testes de resistência de união?

Pesquisador: Rachel de Oliveira Rocha

Área Temática:

Versão: 1

CAAE: 57911616.2.0000.5346

Instituição Proponente: Universidade Federal de Santa Maria/ Pró-Reitoria de Pós-Graduação e

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.670.632

Apresentação do Projeto:

O projeto está vinculado ao Programa de Pós-Graduação em Odontologia e está assim apresentado: "Com a evolução de materiais restauradores com características adesivas se faz importante que estes sejam testados previamente em testes laboratoriais em substratos decíduos. Isto porque existem diferenças na composição e morfologia entre dentes decíduos e permanentes e, além disso, estudos mostram existirem também diferenças na resistência de união ao esmalte e à dentina entre esses dentes. Uma das grandes dificuldades dos estudos laboratoriais de adesão é a obtenção de dentes decíduos hígidos em quantidades suficientes para serem testados. Dessa maneira, o objetivo desse estudo é comparar a resistência de união entre o esmalte e a dentina de dentes decíduos humanos com o esmalte e a dentina de dentes decíduos bovinos através de testes de resistência de união de microcisalhamento e microtração, a fim de confirmar se esses podem ser substitutos para os humanos."

Os dentes humanos serão doados pelo Banco de Dentes Humanos Decíduos da UFSM e os dentes bovinos serão doados pelo Frigorífico Silva.

Pesquisadores apresentam cronograma e orçamento sendo que os custos serão absorvidos pelos

Endereço: Av. Roraima, 1000 - prédio da Reitoria - 2º andar
Bairro: Camobi **CEP:** 97.105-970
UF: RS **Município:** SANTA MARIA
Telefone: (55)3220-9362 **E-mail:** cep.ufsm@gmail.com

pesquisadores.

Objetivo da Pesquisa:
Objetivo geral: investigar se o dente decíduo humano pode ser substituído pelo dente decíduo bovino em testes de resistência de união.

Objetivos específicos:
- Comparar a resistência de união de sistemas adesivos ao esmalte de dentes decíduos bovinos e humanos, por meio do ensaio de microcisalhamento.
- Comparar a resistência de união de sistemas adesivos a dentina de dentes decíduos bovinos e humanos, por meio do ensaio de microtração.

Avaliação dos Riscos e Benefícios:
Sobre os riscos cita-se: "Nao ha riscos ou beneficios diretos da pesquisa para os doadores de dentes deciduos. Os possiveis riscos estao associados aos procedimentos cirurgicos (exodontia) previos a doacao dos dentes ao Banco de Dentes."

Sobre os benefícios cita-se: "Todos os doadores de dentes deciduos contribuem para o desenvolvimento de materiais e tecnicas restauradores, sendo este o beneficio indireto obtido."

Considerando tratar-se de pesquisa laboratorial, riscos e benefícios estão descritos de maneira adequada.

Comentários e Considerações sobre a Pesquisa:
O projeto está bem escrito e organizado. O tema é relevante uma vez que propõe uma alteranativa para realização de pesquisas científicas na área.

Os pesquisadores não apresentam cálculo amostral, no entanto, cada grupo ficará com 7 dentes, o que parece adequado.

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Continuação do Parecer: 1.670.632

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos estão apresentados de maneira adequada.

Pesquisadores propõe dispensa de TCLE uma vez que a pesquisa será desenvolvida com dentes humanos e bovinos doados. É apresentado termo de doação do Banco de Dentes Humanos Decícuos da UFSM.

Recomendações:
Veja no site do CEP - http://w3.ufsm.br/nucleodecomites/index.php/cep - na aba "orientacoes gerais", modelos e orientacoes para apresentacao dos documentos. Acompanhe as orientacoes disponiveis, evite pendencias e agilize a tramitacao do seu projeto.

Conclusões ou Pendências e Lista de Inadequações:
O projeto não apresenta pendências e pode ser aprovado.

Considerações Finais a critério do CEP:

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SANTA MARIA, 10 de Agosto de 2016

Assinado por:
CLAUDEMIR DE QUADROS
(Coordenador)

Endereço: Av. Roraima, 1000 - prédio da Reitoria - 2º andar
Bairro: Camobi **CEP:** 97.105-970
UF: RS **Município:** SANTA MARIA
Telefone: (55)3220-9362 **E-mail:** cep.ufsm@gmail.com

ANEXO B- NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *THE JOURNAL OF ADHESIVE DENTISTRY*

The Journal of Adhesive Dentistry is a bi-monthly journal that publishes scientifically sound articles of interest to practitioners and researchers in the field of adhesion to hard and soft dental tissues. The Journal publishes several types of peer-reviewed original articles:

1. **Clinical and basic science research reports** – based on original research in adhesive dentistry and related topics.
2. **Reviews topics** – on topics related to adhesive dentistry
3. **Short communications** – of original research in adhesive dentistry and related topics. Max. 4 printed pages, including figures and references (max. characters 18,000). High priority will be given to the review of these papers to speed publication.
4a. I**nvited focus articles** – presenting a position or hypothesis on a basic science or clinical subject of relevant related topics. These articles are not intended for the presentation of original results, and the authors of the articles are selected by the Editorial Board.
4b. **Invited commentaries** – critiquing a focus article by addressing the strong and weak points of the focus article. These are selected by the Editorial Board in consultation with the focus article author, and the focus article and the commentaries on it are published in sequence in the same issue of the Journal.
5. **Invited guest editorials** – may periodically be solicited by the Editorial Board.
6. **Proceedings of symposia, workshops, or conferences** – covering topics of relevance to adhesive dentistry and related topics.
7. **Letters to the Editor** – may be submitted to the editorin-chief; these should normally be no more than 500 words in length.

SUBMISSION INSTRUCTIONS
Submission of manuscripts in order of preference:

1.Submission via online submission service (www.manuscriptmanager.com/jadd). Manuscript texts should be uploaded as PC-word files with tables and figures preferably embedded within the PC-word document. A broad range of file formats are acceptable. No paper version required but high resolution photographs or illustrations should be sent to the editorial office (see below). Online submissions are automatically uploaded into the editorial office's reviewer assignment schedule and are therefore processed immediately upon upload.
2.Submission via e-mail as a PC-word document (wintonowycz@quintessenz.de). Illustrations can be attached in any format that
can be opened using Adobe Photoshop, (TIF, GIF, JPG, PSD, EPS etc.) or as Microsoft PowerPoint Documents (ppt). No paper version required but high resolution photographs or illustrations should be sent to the editorial office.
3. One paper copy of the manuscript plus a floppy diskette or CD-ROM (mandatory) containing a PC-word file of the manuscript text, tables and legends. Figures should be included on the disk if possible in any format that can to be opened using Adobe Photoshop, (TIf, GIf, JPG, PSD, EPS etc.) or as a Microsoft PowerPoint Document (ppt)

Mailing address:
Quintessenz Verlags-GmbH, Karin Wintonowycz
The Journal of Adhesive Dentistry,
Ifenpfad 2-4, D–12107 Berlin, Germany

Illustrations that cannot be sent electronically will be scanned at the editorial office so that they can be sent to reviewers via e-mail along with the manuscript to expedite the evaluation process. Resubmitted manuscripts should also be submitted in the above manner. Please note that supplying electronic versions of your tables and illustrations upon resubmission will assure a faster publication time if the manuscript is accepted.

Review/editing of manuscripts. Manuscripts will be reviewed by the editor-in-chief and at least two reviewers with expertise within the scope of the article. The publisher reserves the right to edit accepted manuscripts to fit the space available and to ensure conciseness, clarity, and stylistic consistency, subject to the author's final approval. **Adherence to guidelines.** Manuscripts that are not prep ared in accordance with these guidelines will be returned to the author before review.

MANUSCRIPT PREPARATION

• The Journal will follow as much as possible the recommendations of the International Committee of Medical Journal Editors (Vancouver Group) in regard to preparation of manuscripts and authorship (Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126: 36-47).

• **Title page.** The first page should include the title of the article (descriptive but as concise as possible) and the name, degrees, job title, professional affiliation, contribution to the paper (e.g., idea, hypothesis, experimental design, performed the experiments in partial fulfillment of requirements for a degree, wrote the manuscript, proofread the manuscript, performed a certain test, consulted on and performed statistical evaluation, contributed substantially to discussion, etc.) and full address of all authors. Phone, fax, and e-mail address must also be provided for the corresponding author, who will be assumed to be the first listed author unless otherwise noted. If the paper was presented before an organized group, the name of the organization, location, and date should be included.

• **3-8 keywords**.

• **Structured abstract**. Include a maximum 250-word structured abstract (with headings Purpose, Materials and Methods, Results, Conclusion).

• **Introduction.** Summarize the rationale and purpose of the study, giving only pertinent references. Clearly state the working hypothesis.

• **Materials and Methods.** Present materials and methods in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.

• **Results.** Present results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables or illustrations; emphasize only important observations.

• **Discussion.** Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results section. Relate observations to other relevant studies and point out the implications of the findings and their limitations.

• **Acknowledgments**. Acknowledge persons who have made substantive contributions to the study. Specify grant or other financial support, citing the name of the supporting organization and grant number.

• **Abbreviations**. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

• **Trade names.** Generic terms are to be used when ever possible, but trade names and manufacturer should be included parenthetically at first mention.
• **Clinical Relevance**. Please include a very brief (2 sentences or 3 lines) clinical relevance statement.

REFERENCES

• **All references must be cited** in the text, according to the alphabetical and numerical reference list.
• **The reference list** should appear at the end of the article, in alphabetical and numerical sequence.
• **Do not include unpublished data** or personal communications in the reference list. Cite such references parenthetically in the text and include a date.
• **Avoid using abstracts** as references.
• **Provide complete information** for each reference, including names of all authors. If the reference is part of a book, also include title of the chapter and names of the book‘s editor(s).

Journal reference style:
1. Turp JC, Kowalski CJ, Stohler CS. Treatment- seeking patters of facial pain patients: Many possibilities, limited satisfaction. J Orofacial Pain 1998;12:61-66.
B**ook reference style:**
1. Hannam AG, Langenbach GEJ, Peck CC. Computer simulations of jaw biomechanics. In: McNeill C (ed). Science and Practice of Occlusion. Chicago: Quintessence, 1997:187-194.

ILLUSTRATIONS

• All illustrations must be numbered and cited in the text in order of appearance.
•Submitted figures should meet the following minimum requirements:
– High-resolution images should have a width of 83 mm and 300 dpi (for column size). – Graphics (bar diagrams, schematic representations, drawings) wherever possible should be produced in Adobe Illustrator and saved as AI or EPS files.
– All figures and graphics should be separate files – not embedded in Word or Power Point documents.

Upon article acceptance, high-resolution digital image files must be sent via one of the following ways:
1. As an e-mail attachment, if the files are not excessively large (not more than 10 MB), to our production department: Steinbrueck@quintessenz.de
2. Online File Exchange Tool: Please send your figures with our Online File Exchange Tool. This web tool allows you to upload large files (< 350.0 MB) to our server. Please archive your figures with a maximum size of 350 MB first. Then upload these archives with the following link: http://files.qvnet.de/JAD/, password: IAAD. Please name the archive with your name and article number so we can identify the figures
.
Line drawings–Figures, charts, and graphs should be professionally drawn and lettered large enough to be read after reduction. Good-quality computer-generated laser prints are acceptable (no photocopies); also provide electronic files (eps, ai) if possible. Lines within graphs should be of a single weight unless special emphasis is needed.
Legends–Figure legends should be grouped on a separate sheet and typed double-spaced.

TABLES

• Each table should be logically organized, on a separate sheet, and numbered consecutively.
• The title and footnotes should be typed on the same sheet as the table.

MANDATORY SUBMISSION FORM

The Mandatory Submission Form, signed by all authors, must accompany all submitted manuscripts before they can be reviewed for publication. Electronic submission: scan the signed form and submit as JPG or TIF file.

PERMISSIONS & WAIVERS

• Permission of author and publisher must be obtained for the direct use of material (text, photos, drawings) under copyright that does not belong to the author.
• Waivers must be obtained for photographs showing persons. When such waivers are not supplied, faces will be masked to prevent identification. For clinical studies the approval of the ethics committee must be presented.

PAGE CHARGE

The first 8 printed pages in an article are free of charge. For excess pages, the charge is €140 per printed page. The approximate number of characters on a printed page is approximately 6,800. Please also consider the number and size of illustrations