MURILO BAENA LOPES

Análise estrutural e mecânica de dentes bovinos relacionados a testes de união adesiva

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Doutor em Materiais Dentários.

Orientador: Prof. Dr. Simonides Consani - FOP/UNICAMP

PIRACICABA

2007

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA
Bibliotecário: Marilene Girello – CRB-8ª. / 6159

L881a

Lopes, Murilo Baena.
Análise estrutural e mecânica de dentes bovinos relacionados a testes de união adesiva. / Murilo Baena Lopes. -- Piracicaba, SP: [s.n.], 2007.

Orientador: Simonides Consani.
Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Adesão. 2. Morfologia. 3. Dentes. 4. Materiais dentários. I. Consani, Simonides. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

(mg/fop)

Título em Inglês: Structural and mechanical evaluation of the bovine teeth related to tests of adhesive bond
Palavras-chave em Inglês (Keywords): 1. Adhesion. 2. Morphology. 3. Tooth. 4. Dental materials
Área de Concentração: Materiais Dentários
Titulação: Doutor em Materiais Dentários
Banca Examinadora: Simonides Consani, Lourenço Correr Sobrinho, Mario Alexandre Coelho Sinhoreti, Paulo Henrique dos Santos, Luciana Andrea Sálvio
Data da Defesa: 26-02-2007
Programa de Pós-Graduação: Materiais Dentários

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 26 de Fevereiro de 2007, considerou o candidato MURILO BAENA LOPES aprovado.

PROF. DR. SIMONIDES CONSANI

PROF. DR. PAULO HENRIQUE DOS SANTOS

PROFa. DRa. LUCIANA ANDREA SALVIO

PROF: DR. MARIO ALEXANDRE COELHO SINHORETI

PROF. DR. LOURENÇO CORRER SOBRINHO

Dedico este trabalho a meus pais, José e Joceli, pelo apoio e compreensão e por sempre possibilitarem condições para a formação dos filhos.

Ao meu irmão, Maurício pelo apoio e incentivo.

Agradecimentos Especiais

Ao Prof. Dr. Simonides Consani, Titular da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba – UNICAMP, pela amizade, atenção e dedicação na orientação deste trabalho, e pelo estímulo ao desenvolvimento do espírito crítico.

Ao Prof. Dr. John F. McCabe, Titular da Área de Biomateriais da University of Newcastle upon Tyne, pela orientação e amizade, além de facilitar minha adaptação em um ambiente novo.

Agradecimentos

À Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, na pessoa do seu Diretor Prof. Dr. Francisco Haiter Neto e do Diretor Associado Prof. Dr. Marcelo de Castro Meneghim, pelos bons momentos que aqui passei desde o início da graduação.

Ao Prof. Dr. Mario Fernando de Goes, Titular da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba – UNICAMP, pelo convívio, e conhecimentos transmitidos e críticas construtivas durante o curso.

Ao Prof. Dr. Lourenço Correr Sobrinho, Titular da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba – UNICAMP, pela amizade e orientação científica desde o início de minha formação profissional, incentivando a busca pelo conhecimento.

Ao Prof. Dr. Mário Alexandre Coelho Sinhoreti, Titular da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba – UNICAMP, pela amizade e incentivo na realização de atividades científicas.

À Profª. Drª. Regina Maria Puppin Rontani, Titular da Área Odontopediatria da Faculdade de Odontologia de Piracicaba – UNICAMP, pela possibilidade de expansão do conhecimento em outras áreas.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, pela concessão da bolsa de doutorado e pela bolsa PDEE, que tornou possível a realização do estágio de doutorando na University of Newcastle upon Tyne, Reino Unido.

Ao Engenheiro Mecânico Marcos Blanco Cangiani, Técnico da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba – UNICAMP, pela amizade e constante auxílio na solução das dificuldades.

À Sra. Selma Aparecida Barbosa Segalla, Técnica de Laboratório da Área Materiais Dentários da Faculdade de Odontologia de Piracicaba – UNICAMP, pela amizade, confiança e presteza.

Aos colegas do Programa de Pós-Graduação em Materiais Dentários: Alberto, Alberth, Foguinho, Luciano, Sandrine, Tango, Felipe, Luciana, Flávia, Andréia, Paula, Rogerinho, Giovani, Léo, Mônica, Willian, Marcelo, Ricardo Mineiro, Carinha, Dario, Alysson, Paulinho, Gilberto, Ana Flávia, Juliana, Fabíola, Hugo, Mirela e aos colegas de outras áreas: Regiane, Danna, Lóci, Bruno, Alcides, Fábio, Fykrie, Alinne, Andréia, Guilherme, Fernando, Mirela, Mário, Luís, Larissa, Vanessa, Ramiro, Poliana, Carioca pelos ótimos momentos em que convivemos.

Aos meus colegas de Newcastle upon Tyne: Yan, Guiath, Omar, Megumi, Okba e Atom pela amizade e pelos momentos divertidos no laboratório e fora dele.

MEUS SINCEROS AGRADECIMENTOS.

Anedota Búlgara

Era uma vez um czar naturalista
que caçava homens.
Quando lhe disseram que também se caçam
borboletas e andorinhas,
ficou muito espantado
e achou uma barbaridade...

Carlos Drummond de Andrade

RESUMO

O propósito deste estudo foi: 1 - Comparar os valores de resistência de união sobre esmalte e dentina humanos com os valores obtidos em dentes bovinos, utilizando dois sistemas de união com princípios de atuação distintos; 2 – Comparar as dimensões tubulares e distribuição na dentina humana e bovina; 3 – Comparar duas técnicas (confocal e microscopia óptica) para determinar a microinfiltração em dentes bovinos e humanos para sistemas adesivos. Para o teste de resistência de união, a dentina e o esmalte humano e bovino apresentaram valores equivalentes para o Clearfil Liner Bond 2V. O Scotchbond Multi-Purpose não mostrou diferença estatística em esmalte, porém em dentina o substrato bovino mostrou maiores valores de união que o humano. Quando analisado o diâmetro tubular, a dentina bovina superficial e média mostrou diâmetros estatisticamente maiores que para a dentina humana superficial e média. Não se encontrou diferença estatística entre o diâmetro tubular em dentina humana e bovina em dentina profunda. A densidade dos túbulos dentinários foi estatisticamente maior em dentina humana que em dentina bovina. No teste de microinflitração, quando dente bovino foi utilizado, o Scotchbond apresentou estatisticamente mais infiltração que o Clearfil. Para o dente humano não foi encontrada diferença estatística. Quando escores foram analisados, o Scotchbond não apresentou diferenças para o Clearfil, entretanto este apresentou estatisticamente menos infiltração em dente bovino que em dente humano quando o microscópio óptico foi utilizado. Microscópio confocal mostrou maior especificidade para o corante e limites de microinfiltração mais nítidos. Com o substrato bovino nem sempre se encontrou resultados equivalentes em teste de união com o dente humano. A estrutura dentinária mostrou-se diferente entre dentes humanos e bovinos. O dente bovino não seria o mais adequado para testes de microinfiltração.

ABSTRACT

The purpose of this study was: 1 - To compare shear bond strength values obtained in human enamel and dentin with the values obtained in bovine teeth using two adhesive systems with different actions; 2 – To compared the tubular dimensions and distribution of human dentine and bovine dentine; 3 - To compare two techniques (confocal and optical microscopies) for determining microleakage in human and bovine teeth for adhesive bond systems. For shear bond strength, the bovine and the human dentin and enamel had equivalent values for Clearfil Liner Bond 2V. Scotchbond Multi-Purpose had no statistical differences on enamel, but on dentin, the bovine substrate showed higher bond strength values than the human one. Bovine dentine tubular diameter for superficial and middle dentine was significantly greater than for human superficial and middle dentine. There was no significant difference in tubular diameter between human and bovine deep dentine. The density of dentine tubules is significantly greater in human dentine than in bovine dentine. When bovine teeth were used, Scotchbond showed statistically higher mean penetration than Clearfil. When human teeth were used no difference was found. For microleakage test, when bovine teeth were used, Scotchbond showed statistically higher mean penetration than Clearfil. When human teeth were used no difference was found. When scores were analyzed, Scotchbond showed no difference to Clearfil, however Clearfil showed statistically less infiltration in bovine teeth than in human teeth when optical microscopy was used. Confocal microscopy showed higher sensibility for the stain and clearer leakage limits. The bovine teeth not always show the same pattern of results of human teeth in shear bond test. The dentinal structure showed different between human and bovine substrates. Bovine teeth may be not suitable for adhesive microleakage tests.

SUMÁRIO

1. INTRODUÇÃO GERAL

Desde o começo da era adesiva com a publicação de um artigo por (Buonocore, 1955), centenas de estudos vem sendo realizados no intuito de obter melhoras nos sistemas de união. O desenvolvimento de materiais restauradores adesivos têm resultado em uma crescente atenção para a estrutura dentinária, o conhecimento detalhado dessa estrutura se tornou essencial para a interpretação dos resultados obtidos em investigações na interação do substrato dentário-adesivo (Rueggeberg, 1991;Marshall, 1993).

Para avaliar a eficiência dos materiais restauradores *in vitro*, pequisadores têm usado vários substratos para simular a condição clínica. Esmalte e dentina humana são geralmente os substratos de escolha para avaliar força de resistência de união adesiva (Mellberg, 1992). Muitos estudos *in vitro* vem utilizando dentes humanos extraídos, entretanto, estes são às vezes difíceis de se obter devido ao progresso da odontologia preventiva e as técnicas restauradoras mais conservadoras que surgiram durante nas últimas décadas (Nakamichi *et al.*, 1983). Por isso a importância de se considerar substratos alternativos como dentes de outros mamíferos, que na maioria das vezes são histologicamente e morfologicamente similares aos dentes humanos (Weichert&Presch, 1975). Pesquisadores têm utilizado dentes bovinos, ovinos, eqüinos ou suínos como fonte para prover quantidades de material padronizado para seus estudos. (Fusayama *et al.*, 1979) testaram a possibilidade de utilizar o marfim como substituto para dentina bovina em teste de resistência à tração adesiva não obtendo resultados similares ao substrato humano. Esses substratos alternativos vêm sendo utilizados em estudos sobre lesão de cárie incipiente (Pearce, 1983), taxas de progressão de lesões de cárie, (Featherstone&Mellberg, 1981) microdureza e estudos de profundidade de lesão (Arends *et al.*, 1980), além de estudos de adesão de materiais adesivos (Nakamichi, 1982). Propriedades físicas seguidas por ataque ácido (Groenhuis *et al.*, 1980) ou abrasão (Putt *et al.*, 1980) foram também estudados.

O substituto mais adequado para uma padronização das mensurações da força de união a dentina humana é a superfície vestibular dos terceiros molares humanos (Olsson *et al.*, 1993). Esses dentes têm superfície suficiente para realização de testes de união além de normalmente serem encontrados intactos.(Schilke *et al.*, 2000) Como um substituto para o dente humano, os incisivos permanentes bovinos são os mais freqüentes utilizados em testes de adesão entre materiais restauradores e tecido duro dental.(Schilke *et al*, 2000) Em testes adesivos, espécimens de dentina bovina coronal e dentina bovina radicular tem sido utilizados.(Burrow *et al.*, 1996;Schilke *et al.*, 1999) O tamanho e a disponibilidade de obtenção fazem dos incisivos bovinos o substrato alternativo de escolha para realização de testes de união adesiva. (Burrow *et al*, 1996;Reeves *et al.*, 1995) Entretanto, existem preocupações a respeito de até onde os dados obtidos em dentes bovinos são válidos para dentes humanos e qual a validade desses testes em situações clínicas. (Nakamichi *et al*, 1983;Reeves *et al*, 1995). É possível também que existam diferenças químicas entre os dois tipos de substratos, e isso talvez seja de importância significante. (Sydney-Zax *et al.*, 1991) reportaram que o esmalte bovino não erupcionado tem uma concentração de carbonato mais elevada que o esmalte humano, indicando que o dente bovino talvez seja mais susceptível ao ataque ácido devido as variações na hidroxiapatita.

Apesar dos avanços da odontologia e de melhor entendimento das características do substrato dentário, os quais promoveram decréscimo no grau de infiltração marginal (Pazinatto *et al.*, 2003), os compósitos odontológicos continuam a contrair durante a reação de polimerização e a ter coeficiente de expansão linear diferente da dentina e esmalte, contribuindo para a existência de microinfilração marginal (Nelsen *et al.*, 1952).

Em ordem para predizer a performance clínica do esmalte e dentina unidos com material restaurador adesivo, geralmente são realizados três tipos de testes *in vitro*: observação morfológica, estudos de microinfiltração e estudos de força de união. Testes de microinfiltração, o qual é definido como a passagem indetectável de fluidos e bactérias entre o material restaurador e o dente preparado (Kidd, 1976), é o teste mais utilizado em laboratório para estudar os mecanismos que podem minimizar ou eliminar a infiltração ao redor da restauração (Pazinatto *et al*, 2003).

Com isso, este trabalho tenta conseguir mais informações a respeito da estrutura dentária, com ênfase à dentina, para melhor entendimento das interações material-substrato, além de comparar o substrato humano com o alternativo bovino.

Desta maneira, os objetivos desta tese[1], divididos em capítulos foram: 1 - Avaliar as diferenças comportamentais em testes de união adesiva entre os substratos dentários humano e bovino; 2 – Comparar a estrutura tubular entre dentes humanos e bovinos a diferentes profundidades; 3 - Comparar a união entre os substratos humano e bovino em teste de microinfiltração utilizando dois tipos de microscopia.

[1] Este trabalho foi realizado no formato alternativo, com base na deliberação da Comissão Central de Pós-graduação (CCPG) da Universidade Estadual de Campinas (Unicamp) nº 001/98

2. CAPÍTULO 1

Dental Materials

Comparative study of the dental substrate used in shear bond strength tests

Estudo comparativo do tipo de substrato dental utilizado em testes de resistência de união ao cisalhamento

Artigo publicado na Pesquisa Odontológica Brasileira, v.17, n.2, 2003, p.171-5

ABSTRACT: The purpose of this study was to compare shear bond strength values obtained in human enamel and dentin with the values obtained in bovine teeth using two adhesive systems with different actions. Forty human half-crowns teeth and forty bovine crowns teeth were flattened to a minimum plain area of 5mm in diameter. The samples were divided in four groups of 20 specimens each: 1) human enamel; 2) bovine enamel; 3) human dentin; 4) bovine dentin. The samples of each group were divided in 2 subgroups of 10 samples each, according to the adhesive system used: 1) Scotchbond Multi Purpose (SBMP); and 2) Clearfil Liner Bond 2V (CLB2V) applied according to the manufacturer's recommendations. After, restorations of Z100 composite with cylindrical shape (4mm diameter X 5mm height) were made using a metallic mold to submit the samples to shear bond test on Instron universal testing machine, at crosshead speed of 0.5 mm/min. The data were submitted to ANOVA and Tukey's test (5%). In enamel, there was no statistical difference between bovine and human teeth for SBMP (7.36 MPa, human; 8.24 MPa, bovine), nor for CLB2V (10.01 MPa, human; 7.95, bovine). In dentin, it was verified that SBMP showed statistically lower mean on human dentin (5.01 MPa), than on bovine dentin (11.74 MPa). For CLB2V, there was no statistical difference between human (7.43 MPa) and bovine (9.27 MPa) substrates.
DESCRIPTORS: dentin-bonding agents; dentin; dental enamel.

RESUMO: O propósito deste estudo foi comparar os valores de resistência de união sobre esmalte e dentina humanos com os valores obtidos em dentes bovinos, utilizando dois sistemas de união com princípios de atuação distintos. Para isto, desgastou-se 40 meias coroas dentais humanas e 40 coroas bovinas, até obter uma área plana de pelo menos 5 mm de diâmetro. As 80 amostras foram separadas em 4 grupos de 20 amostras cada, sendo: 1) dente humano em esmalte, 2) dente bovino em esmalte, 3) dente humano em dentina, 4) dente bovino em dentina. As amostras de cada grupo foram divididas em 2 subgrupos de 10 amostras cada, de acordo com o sistema de união utilizado: 1) Scocthbond Multi Uso (SBMU); e 2) Clearfil Liner Bond 2V (CLB2V). Após, confeccionou-se um cilindro do compósito Z-100 (4mm de diâmetro X 5mm de altura) utilizando uma matriz bipartida, para submeter os corpos-de-prova ao ensaio de cisalhamento numa máquina de ensaio Instron com velocidade de 0,5mm/min. Os dados foram submetidos à análise de variância e as médias ao teste de Tukey (5%). Em esmalte, não se verificou diferença estatística entre os dentes humanos e bovinos para os materiais SBMU (7,36 MPa, humano; e 7,24 MPa, bovino) e CLB2V (10,01 MPa, humano; e 7,95 MPa, bovino). Verificou-se que o SBMU apresentou média estatísticamente inferior em dentina humana (7,01 MPa), quando comparado à dentina bovina (11,74 MPa). Para o material CLB2V, não houve diferença estatística entre os substratos humano (7,43 MPa) e bovino (9,27 MPa).
DESCRITORES: adesivos dentinários; dentina; esmalte dentário.

INTRODUCTION

Since the acid-etch technique was first introduced in dentistry by Buonocore[2], phosphoric acid has been routinely used to roughen the enamel surface in order to create micromechanical retention with resin composites. The success obtained with enamel inspired its use on dentin surface as well. However, with the use of early hydrophobic resins, acid treatment of dentin did not produce bond strength similar to that obtained on enamel surfaces[13].

After that, adhesive systems incorporating acid-etch and hydrophilic monomers have been developed. The enamel and dentin acid-etch could be done simultaneously[8] and there was an increase in the adhesive bond strength. With the new adhesive systems, a “primer” became necessary after the acid-etching and before the bonding agent. It increased the wettability of dentin surface for the penetration of the adhesive agent.

Recently, "one-bottle" adhesive systems were introduced in dentistry. They combined the "primer" and the adhesive functions into a sole solution. These systems require at least two layers of the solution and a previously enamel and dentin acid-etching.

Nowadays, "self-etching" adhesive systems have currently combined the tooth surface etching and primer steps to treat enamel and dentin, which are demineralized allowing the adhesive to infiltrate. They have been developed by raising the concentration of the acid adhesive monomers from their original 5-6% concentration to 20% or more[11,16]. By dissolving the acid monomers in 2-

hydroxyethylmethacrylate (HEMA) and because of its low pH, it resulted in a satisfactory condition system[9,19].

To evaluate the adhesive bond strength, plenty of *in vitro* research has been developed using extracted human teeth, which are, however, more and more difficult because of preventive dentistry advances. Therefore, it became necessary to look for an alternative substrate. As mammalian teeth are histologically and morphologically similar[17], investigators have turned to teeth from bovine, ovine, equine, or swine to provide quantities of standardized material for their studies. Schilke *et al*.[14] verified that there were no statistically significant differences neither in the number of tubules per mm^2 nor in their diameters in corresponding coronal dentine layers of human deciduous and permanent molars, and of bovine central incisor.

The size and availability make bovine incisors preferable for bond strength research[4,12]. However, there is some concern about whether data obtained from bovine teeth can be applied to human teeth and valid in a clinical situation[10,12].

So, this study searches for more information about the alternative substrate for human teeth. Therefore, a comparison was made about shear bond strength between human and bovine enamel and dentin, using two different adhesive systems with different mechanism of action.

MATERIALS AND METHODS

Forty fresh extracted, non carious human molars and eighty bovine incisors were obtained and stored in 0.9% sodium hypochlorite for at maximum 90 days. The roots of the teeth were removed. Human crowns have been sectioned at vestibule-buccal direction, in a total of forty half-crowns. The crowns were then embedded with the vestibule (bovine) or vestibule or buccal (human) surface exposed in plastic tubes with self-cured acrylic resin. The samples were assigned into 4 groups: 1) human enamel, 2) bovine enamel, 3) human dentin, and 4) bovine dentin; with 20 specimens in each group. The vestibule or proximal surfaces were ground wet in a polishing machine (APL-4 Arotec) with 180 grit, followed by 400 and 600 grit silicon carbide paper to create a 5 mm diameter flat surface on enamel or dentin. To delimitate the testing area, a circular adhesive tape with a central orifice of 4 mm in diameter was applied.

The samples of each group were assigned into two subgroups of 10 specimens, according to the adhesive system used: 1) Scotchbond Multi-purpose (3M Dental Products Division, St. Paul, MN, USA), 2) Clearfil Liner Bond 2V (Kuraray CO. LTD, Osaka, Japan).

After these superficial treatments, a stainless steel mould (4 mm in diameter and 5 mm in height) was placed against the specimen to receive the filling material (Z-100, 3M Dental Products Division, St. Paul, MN, USA). The resin composite was inserted in three increments of equal height; each one of them was light-cured (XL–3000, 3M Dental Products Division, St. Paul, MN, USA) for 40s, with a light intensity of 530 mW/cm^2. After mould removal, the specimens were stored in distillated water at 37^{o}C for 24 hours. After this period, the specimens were positioned in a universal testing machine (Instron, model 4411) to be submitted to a shear bond strength test, performed at a speed of 0.5 mm/min until breakdown. A stainless steel strip involved the resin cylinder and was fixed in the inferior mordant. A metal jig, which involved the specimen, was fixed in the superior mordant. The obtained data (Kgf/cm^2) were transformed in MPa and analyzed with ANOVA and Tukey's test (p = 0.05).

RESULTS

The shear bond strength results were submitted to ANOVA with factorial schema. The factors were: the substrate, analyzed for their nature (enamel or dentin); the origin, analyzed for animal species (human or bovine). Two analyses were carried out: one for Clearfil Liner Bond 2V and another for Scotchbond Multi-purpose. This was done because the purpose of the study was not to evaluate the two adhesive systems themselves, but compare the adhesive systems with dissimilar mechanisms of action in different substrate. The means were submitted to Tukey's test with 5% of significance and they are presented on Tables 1 and 2.

Table 1 – Mean and standard deviation of shear bond strength (MPa) for Clearfil Liner Bond 2V, on human and bovine enamel and dentin.

	Enamel	5%	Dentin	5%
Human	10.01 a	(3.22)	7.43 a	(2.09)
Bovine	7.95 a	(1.98)	9.27 a	(2.69)

Means with same letters on column indicate no significant difference for Tukey's test (5%). Figures in parentheses are standard deviations.

Table 1 shows for Clearfil Liner Bond 2V, no significant statistical difference ($p>0.05$) between bovine and human teeth for both enamel and dentin.

Table 2 – Mean and standard deviation of shear bond strength (MPa) for Scotchbond Multi-purpose, on human and bovine enamel and dentin.

	Enamel	5%	Dentin	5%
Human	7.36 a	(1.58)	7.01 a	(2.26)
Bovine	8.24 a	(2.47)	11.74 b	(3.78)

Means with same letters on column indicate no significant difference for Tukey's test (5%). Figures in parentheses are standard deviations.

Table 2 shows for Scotchbond Multi-purpose, no significant statistical difference ($p>0.05$) between bovine and human teeth for enamel. However, on dentin, the bovine tooth shear bond mean was higher than on the human one and there was some statistical difference ($p<0.05$).

DISCUSSION

The search for an alternative substrate to human teeth for studies on bond strength and microleakage has increased the interest of some researchers.

The strength of the adhesive bonds between restorative materials and dentin is affected by the number of dentinal tubules per mm^2 and by their diameter, as well as the relative amount of intratubular and intertubular dentin[10]. According to Nakamichi *et al.*, a sufficient area of substrate could be obtained in different dentin depth of bovine incisors, but only the superficial layer could be considered a substitute to human dentin. The dentin in bovine incisors presents larger dentinal tubules and more porous on intertubular dentin than the human molars[12].

Bovine enamel is more porous than human enamel, so it presents a higher diffusion and rate of progress of artificial carious lesion[5,6,7]. Besides, it presents thinner crystallites than human enamel and it has different structure among the prisms[18]. Edmunds *et al.*[5] using bacterial culture and acid gel studied artificial caries in bovine, equine, and ovine enamel. They verified that the lesions depth in these animal teeth were almost two times bigger than in the human teeth.

The enamel rich in carbonate was found to be particularly vulnerable to acid attack[19]. According to Sydney-Zax *et al.*[15], the carbonate concentration on bovine enamel was higher than in the human one. It is probable that the same occurs with dentin.

This study found higher shear bond strength for bovine substrate when SBMP was used. As bovine substrate presents lower mineral quantity and higher carbonate concentration, it is more susceptible to acid-etching, in other words, on the same period of time, the enamel crystals dissolution, the dentinal tubules open and inter and intratubular demineralization are higher to bovine tooth than to the human one. To enamel, despite the statistical analysis that showed no difference between bovine (8.24 MPa) and human (7.36 MPa) substrate, it could be verified high values to bovine enamel. There was some statistical difference to dentin, with higher values to bovine (11.74 MPa) than to human (7.01 MPa) dentin. Maybe these results were obtained because it is easier to the adhesive system to penetrate into the bovine demineralized dentin, because of the higher dentinal tubules quantity and the bigger diameter. Their longer and thicker tags may induce to higher bond strength values on shear or traction tests.

With Clearfil Liner Bond 2V, there was no difference between human and bovine substrates, either enamel or dentin. But, Nakamichi *et al.* verified higher bond strength to the human enamel than the bovine one. In this study, human enamel also presented a few higher values, but without statistical difference. Maybe these results were because self-etching systems do not need a previous acid-etching with phosphoric acid. So, the acid monomers of these systems will act on the enamel surface, demineralizing and penetrating it. But, it is necessary a close contact with dental surface. According to Yu & Chang[20], bovine enamel presents lower superficial energy than the human one. This could explain why Clearfil Liner Bond 2V showed lower results on bovine enamel.

To Clearfil Liner Bond 2V, on dentin, there were a few high values for bovine tooth compared with the human one, although without statistical difference. The Scotchbond Multi-Purpose had the same behavior, nevertheless it presented statistical difference. An explanation to these results would be that acid-etching before the bond agent application could permit a higher resin penetration on dentinal tubules and on peri and intratubular dentin. With Clearfil Liner Bond 2V system, the resin penetration would be less intense on both substrates. However, when analyzing bond strength values, they are similar to Scotchbond Multi-Purpose ones. Nevertheless, when a shear force was applied, a thicker hybrid layer with larger and longer resin tags, similar to Scotchbond Multi-Purpose one, may possibly make the difference between the substrates to one system and not to the other.

This study verified that when bovine teeth were used instead of human teeth on laboratory tests of bond strength, it is necessary to be careful because the results will not always be proportional between the two substrates.

CONCLUSION

1 – To Clearfil Liner Bond 2V, the bovine and the human dentin and enamel were equivalent on shear bond strength values;

2 – Scotchbond Multi-Purpose had no statistical differences on enamel, but on dentin, the bovine substrate showed higher bond strength values than the human one;

3 – The bovine teeth in the place of human teeth in laboratory tests of shear bond strength seem to be partially valid because the obtained values were not always proportional to the two substrates; this depends on the adhesive system used.

REFERENCES

1 – Arends J, Jongebloed WL. Crystallite dimensions of enamel. J Biol Buccale. 1978 Sep;6(3):161-71.

2 – Buonocore MG. A Simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955 Dec;34(6):849-853.

3 – Buonocore MG, Matsui A, Gwinett AJ. Penetration of resin dental materials into enamel surfaces with reference to bonding. Arch Oral Biol. 1968 Jan;13(1):61-70.

4 - Burrow MF, Sano H, Nakajima M, Harada N, Tagami J. Bond strength to crown and root dentin. Am J Dent. 1996 Oct;9(5):223-9.

5 – Edmunds DH, Whittaker DK, Green RM. Suitability of human, bovine, equine, and ovine tooth enamel for studies of artificial bacterial carious lesions. Caries Res. 1988;22(6):327-36.

6 – Featherstone JD, Mellberg JR. Relative rates of progress of artificial lesions in bovine, ovine and human enamel. Caries Res. 1981;15(1):109-14.

7 – Flim GJ, Arends J. Diffusion of ^{45}Ca in bovine enamel. Calcif Tissue Res. 1977 Dec 14;24(1):59-64. apud J Dent Res. 1992 Apr;71 Spec No:913-9. Review.

8 – Fusayama T, Nakamura M, Kurosaki N, Iwaku M. Non-pressure adhesion of a new adhesive restorative resin. J Dent Res. 1979 Apr;58(4):1364-70.

9 – Miyazaki M, Hirohata N, Takagaki K, Onose H, Moore BK. Influence of self-etching primer drying time on enamel bond strength of resin composites. J Dent. 1999 Mar;27(3):203-7.

10 - Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res. 1983 Oct;62(10):1076-81.

11 – Pashley DH, Sano H, Yoshiama M, Carvalho M. Dentin, a dynamic bonding substrate: The effects of dentin variables on resin adhesion. *In*: Shimono M, Maeda T, Suda H, Takahashi K. Dentin/Pulp Complex. Tokyo: Quintessence, 1996:11-21.

12 - Reeves GW, Fitchie JG, Hembree JH Jr, Puckett AD. Microleakage of new dentin bonding systems using human and bovine teeth. Oper Dent. 1995 Nov-Dec;20(6):230-5.

13 – Rider M, Tanner AN, Kenny B. Investigation of adhesive properties of dental composite materials using an improved tensile test procedure and scanning electron microscopy. J Dent Res. 1977 Apr;56(4):368-78.

14 - Schilke R, Lisson JA, Bauss O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol. 2000 May;45(5):355-61.

15 – Sydney-Zax M, Mayer I, Deutsch D. Carbonate content in developing human and bovine enamel. J Dent Res. 1991 May;70(5):913-6.

16 – Watanabe I, Nakabayashi N, Pashley DH. Bonding to ground dentin by a phenyl-P self-etching primer. J Dent Res. 1994 Jun;73(6):1212-20.

17 - Weichert CK, Presch W. Elements of chordate Anatomy 4th edition. New York: McfGraw-Hill Company 1975.

18 – Whittaker DK, Green RM, Edmunds DH. Structural characteristics of bovine enamel. [abstract 208]. J Dent Res. 1983 Apr;62(4):439.

19 – Yoshiyama M, Matsuo T, Ebisu S, Pashley D. Regional bond strengths of self-etching/self-priming adhesive systems. J Dent. 1998 Sep;26(7):609-16.

20 – Yu KC, Chang R. Adhesive restorative dental materials II. Approaches to achieves adhesion. National Institute of Dental Research: U. S. Department of Health, Education and Welfare. 1966, p.103-113 apud J Dent Res. 1983 Oct;62(10):1076-81.

3. CAPÍTULO 2

Comparative study of tubular diameter and quantity for human and bovine dentine at different depths

Running title: Structure of bovine and human dentine

Artigo enviado para Archives of Oral Biology

Keywords: dental morphology – dentinal tubules – adhesive dentistry

ABSTRACT

This study compared the tubular dimensions and distribution of human dentine and bovine dentine. Ten human molars and ten bovine incisors were ground with a turbine hand-piece to obtain three sections of different dentine depth (superficial, middle and deep). The specimens were coated with gold in a sputtering machine to be observed using SEM. Three photomicrographs were recorded randomly for each dentine depth. The number of tubules was counted and the diameter of 5 tubules selected randomly were measured for each photomicrograph. The results were submitted to analysis of variance and Tukey's test (5%). Bovine superficial dentine tubules (4.21 µm) had a significantly greater diameter than middle dentine (3.98µm) and deep dentine (3.21 µm). Human superficial dentine tubule diameter (2.42 µm) was significantly less than in the deep dentine (2.99 µm) and middle dentine (2.94 µm), which do not differ significantly from each other. The number of tubules per mm^2, independent of region, was significantly greater for human dentine (22,329) than bovine dentine (15,964). There was a clear difference in tubule structure and morphology between human and bovine dentine.

INTRODUCTION

Nowadays, with advances in dentistry, most restorative procedures involve the use of an adhesive step. To evaluate the efficacy of adhesive materials *in vitro*, researchers have used various substrates to simulate the clinical condition. Human enamel and dentine are generally the substrate of choice(Mellberg, 1992) for studies to evaluate adhesive bond strength. Many *in vitro* research has been carried out using extracted human teeth, which are sometimes difficult to obtain for various reasons. Therefore, it has become necessary to consider alternative substrates. As most mammalian teeth are histologically and morphologically similar,(Weichert&Presch, 1975) investigators have turned to teeth from bovine, ovine, equine, or swine sources to provide quantities of standardized material for their studies. These alternative substrates have been used in studies of incipient carious lesions,(Pearce, 1983) rates of progress of artificial carious lesions(Featherstone&Mellberg, 1981), microhardness and lesion depth studies(Arends *et al*, 1980) and in studies of adhesion of resins to enamel surfaces.(Nakamichi, 1982) In addition, a number of studies on the organic and inorganic components of these animal enamels have been carried out(Shearer *et al.*, 1980;Mellberg&Loertscher, 1974). Physical properties following etching(Groenhuis *et al*, 1980) or abrasion(Putt *et al*, 1980) have also been investigated.(Edmunds *et al.*, 1988) Their size and availability make bovine incisors potentially very useful for bond strength research.(Burrow *et al*, 1996;Reeves *et al*, 1995) However, there is some concern about whether data obtained from bovine teeth can be applied to human teeth and therefore about their validity in a clinical situation.(Nakamichi *et al*, 1983;Reeves *et al*, 1995)

Dentine microstructures and properties are the principal determinants of many procedures in restorative dentistry. Although significant progress in restorative and preventive dentistry has occurred over the past several decades based on the increased understanding of the caries process and introduction of increasingly effective bonding techniques, a key problem which remains is our lack of detailed understanding of dentine itself.(Marshall *et al.*, 1997) This is because dentine is a complex hydrated biological composite structure for which only limited structure-property relationships are available.(Marshall *et al*, 1997)

The strength of the adhesive bonds formed between restorative materials and dentine is affected by the number and concentration of dentinal tubules per mm^2 and their diameter, as well as the relative amount of intratubular and intertubular dentine.(Marshall *et al*, 1997;Pashley&Carvalho, 1997) For example, it is know that more intertubular dentine is present in the crown than in the root. Species differences may also exist for both crown and root dentine.(Mjor&Nordahl, 1996) Schilke *et al*.(Schilke *et al*, 2000) indicated that there were no differences in the diameter, number or concentration of tubules in corresponding coronal dentine layers of human deciduous and permanent molars and bovine central incisors. In the study of Tagami *et al.*(Tagami *et al.*, 1990) an increase in the permeability of dentine when the depth increased was reported. This was noted for both human and bovine dentine. Bovine incisor dentine possessed large dentinal tubules and high microporosity, which made it similar to human molar root dentine.

It is possible that chemical differences between different types of teeth may also be significant. For example, Sydney-Zax *et al.*(Sydney-Zax *et al*, 1991) reported that unerupted mature bovine enamel was slightly higher in carbonate concentration than human enamel, indicating that bovine teeth may be more susceptible to acid attack due to the variations in the hydroxyapatite lattice.

In order to establish a meaningful comparison of human and bovine dentine, this study compared the diameter and the number of tubules per millimetre square between the two substrates. The hypothesis to be tested was that bovine teeth are an acceptable substrate for use in *in-vitro* testing of dental adhesive and restorative materials due to the similarities in morphology of the two types of teeth.

MATERIAL AND METHODS

The study utilized 10 non-carious human molar teeth extracted for orthodontic reasons from young patients (18-25 years-old) after the approval of the Research Ethics Committee of the School of Dentistry of Piracicaba (register number 106/2004) and 10 bovine incisors teeth obtained from an abattoir, which had their periodontal ligament removed with a 5-6 Gracey curette. The teeth were stored in 0.9% sodium hypochlorite for a maximum of 90 days. The roots were removed using a saw (Model 650, South Bay Technology) and the crowns were ground with a turbine hand-piece using FG4138, FG4138F and FG4138FF burs (MKS – Brazil) to obtain three sections of dentine at different depths having a tubular orientation, perpendicular to the observed surfaces. Samples of three different depths (superficial, middle and deep of dentine) were obtained for each tooth. The specimens were conditioned with 37% phosphoric acid to cause opening of the dentinal tubules, (20s for human dentine and 40s for bovine dentine). The specimens were cleaned in running flow water and ultrasonically. The specimens were metalized (Balzers, SCD-050, Liechtenstein) in order to enable imaging with a scanning electronic microscope (SEM). Nine photomicrographs were recorded per sample; three random photomicrographs being recorded for each dentine depth. On each photomicrograph, five random tubule diameters were measured and the number of tubules per unit area was counted (Figure 1). The means of tubule diameter from each specimen and the number of tubules per unit area were submitted to analysis of variance and Tukey's test at a 5% level of significance.

RESULTS

Table 1 shows the dentine tubule diameters for human and bovine teeth in superficial, middle and deep dentine. The superficial bovine dentine tubule diameter (4.21 μm) and middle dentine (3.98 μm) was greater than the deep dentine (3.21 μm) tubule diameters. The human superficial dentine tubule diameter (2.42 μm) was significantly less than in the deep dentine (2.99 μm) and middle dentine (2.94 μm), which do not differ significantly from each other. The diameter of dentine tubules was statistically greater in bovine superficial (4.21 μm) and middle (3.98 μm) dentine than in human superficial (2.42 μm) and middle (2.92 μm) dentine respectively. The bovine deep dentine (3.14 μm) and human deep dentine (2.99 μm) tubule diameters were not significantly different.

Table 2 shows the concentration of dentinal tubules (per mm^2) for the three depths of bovine and human teeth. The number of bovine superficial dentine tubules per mm^2 was less than in the middle dentine and deep dentine, which also differ significantly from each other. The concentration of tubules in human deep dentine was greater than in middle dentine and superficial dentine, which also differ from each other. The concentration of tubules in human teeth was greater than in bovine teeth at all dentine depths.

DISCUSSION

Human teeth must be considered the most appropriate hard tissue substrate for *in situ* studies from the perspective of clinical relevance(Zero, 1995). However, human teeth are of a highly variable composition due to genetic influences, environmental conditions and age. As for enamel is concerned, bovine enamel has a more uniform composition than human enamel, and thus provides a less variable substrate for research purposes. However, bovine enamel is more porous and demineralises faster(Mellberg, 1992;Zero, 1995) than human enamel. Edmunds *et al.*(Edmunds *et al*, 1988) concluded from their work on human teeth that the considerable variability in structure of human surface enamel may result in variation in caries susceptibility among individuals. Furthermore, Fonseca *et al.*(Fonseca *et al.*, 2004) found that bovine and swine teeth have lower values of radio density when compared to human teeth. This confirms in a indirect way that the mineral composition of human and bovine teeth is different.

Among the different groups of teeth, incisors are preferred for *in vitro* studies when using bovine teeth due to their size and relatively flat surface.(Mellberg, 1992) When using human teeth, it is difficult to obtain non-carious incisors and therefore molars are preferred.

In this study, a different structure in the bovine dentinal tubules was found compared to human dentine. This anomalous structure found in many of the specimens analysed has the appearance of a tubule conjunction, resulting in a wider tubular structure (Figures 2 and 3). Moriwaki *et al.*(Moriwaki *et al.*, 1968) reported that the large crystals grains and the lattice defects found in bovine enamel were the result of the rapid development of bovine teeth during tooth formation before and after eruption. This may happen with dentine also and may explain the origin of these bovine dentine tubule conglomerates.

Yu and Chang(Yu&Chang, 1966) reported that the critical surface tension of bovine teeth is lower than that for human teeth, resulting in slightly lower bond strength of adhesives to bovine enamel and dentine.

Causton(Causton, 1984), McCabe & Rusby(McCabe&Rusby, 1992) and Susuki & Finger(Suzuki&Finger, 1988) showed the existence of lower values of bond strength on human dentine near to the pulp when compared to superficial dentine. Öilo & Olsson(Oilo&Olsson, 1990) showed that a higher bond strength to dentine from a buccal area than from an occlusal area is obtained with human third molars. Hence, there is much circumstantial evidence of a variation of dentine structure with depths. This study showed that the tubular diameter in superficial and middle bovine dentine was significantly greater than for human superficial and middle dentine. For deep dentine no difference between bovine and human dentin tubule diameter was found. The values obtained indicate that the morphology of dentinal tubules is different for the two substrates with human dentine having a larger diameter of dentine tubules near the pulp and smaller diameter near the cement-enamel junction. Bovine dentine seems to be the opposite, with greater tubules diameter near the cement-enamel junction and smaller diameter near the pulp.

When the number of tubules per mm^2 was compared, human dentine had a greater concentration of tubules compared to bovine dentine, independent of the region depth. In the same substrate group (human or bovine), deep dentine had a greater concentration of tubules than middle or superficial dentine.

The differences observed in the present study may explain the differences in behaviour of human and bovine substrates when using different adhesive materials. For example, Lopes *et al.*(Lopes *et al.*, 2003) showed significant differences for shear bond strength when using the same total etch adhesive system between human and bovine dentine.

This study showed some morphological differences between human and bovine dentine tubules, which may influence adhesive test results. The presence of larger diameter conglomerate tubules in bovine dentine may also be a significant factor. Therefore, it is necessary to be cautious when using bovine dentine for the evaluation of adhesives.

CONCLUSION

1 – Bovine dentine tubular diameter for superficial and middle dentine was significantly greater than for human superficial and middle dentine.

2 – There was no significant difference in tubular diameter between human and bovine deep dentine.

3 – The density of dentine tubules is significantly greater in human dentine than in bovine dentine.

4 – Human dentine tubular diameter for deep and middle dentine was significantly greter than for superficial dentine. The density of tubules was significantly greater for deep dentine, followed by middle and superficial dentine, which differs from each other.

5 - Bovine dentine tubular diameter for superficial and middle dentine was significantly greter than for deep dentine. The density of tubules was significantly greater for deep dentine, followed by middle and superficial dentine, which differs from each other.

Acknowledgment

Grateful acknowledgement is made to CAPES for its support in this research - BEX: 3427/04-8.

REFERENCES

1. Mellberg JR. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity in situ. J Dent Res 1992;71 Spec No:913-9.

2. Weichert CK, Presch W. Elements of chordate anatomy. New York ; London: McGraw-Hill; 1975.

3. Pearce EI. A microradiographic and chemical comparison of in vitro systems for the simulation of incipient caries in abraded bovine enamel. J Dent Res 1983;62(9):969-74.

4. Featherstone JD, Mellberg JR. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. Caries Res 1981;15(1):109-14.

5. Arends J, Schuthof J, Jongebloed WG. Lesion depth and microhardness indentations on artificial white spot lesions. Caries Res 1980;14(4):190-5.

6. Nakamichi I. [Adhesion of various dental restorative materials to human and bovine teeth (author's transl)]. Kokubyo Gakkai Zasshi 1982;49(1):31-40.

7. Shearer TR, Johnson JR, DeSart DJ. Cadmium gradient in human and bovine enamel. J Dent Res 1980;59(6):1072.

8. Mellberg JR, Loertscher KL. Comparison of in vitro fluoride uptake by human and bovine enamel from acidulated phosphate-fluoride solutions. J Dent Res 1974;53(1):64-7.

9. Groenhuis RA, Jongebloed WL, ten Bosch JJ. Surface roughness of acid-etched and demineralized bovine enamel measured by a laser speckle method. Caries Res 1980;14(5):333-40.

10. Putt MS, Kleber CJ, Muhler JC. A comparison of the polishing properties of human and bovine enamel. J Dent Res 1980;59(7):1177.

11. Edmunds DH, Whittaker DK, Green RM. Suitability of human, bovine, equine, and ovine tooth enamel for studies of artificial bacterial carious lesions. Caries Res 1988;22(6):327-36.

12. Burrow MF, Sano H, Nakajima M, Harada N, Tagami J. Bond strength to crown and root dentin. Am J Dent 1996;9(5):223-9.

13. Reeves GW, Fitchie JG, Hembree JH, Jr., Puckett AD. Microleakage of new dentin bonding systems using human and bovine teeth. Oper Dent 1995;20(6):230-5.

14. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res 1983;62(10):1076-81.

15. Marshall GW, Jr., Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997;25(6):441-58.

16. Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. J Dent 1997;25(5):355-72.

17. Mjor IA, Nordahl I. The density and branching of dentinal tubules in human teeth. Arch Oral Biol 1996;41(5):401-12.

18. Schilke R, Lisson JA, Bauss O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol 2000;45(5):355-61.

19. Tagami J, Tao L, Pashley DH. Correlation among dentin depth, permeability, and bond strength of adhesive resins. Dent Mater 1990;6(1):45-50.

20. Sydney-Zax M, Mayer I, Deutsch D. Carbonate content in developing human and bovine enamel. J Dent Res 1991;70(5):913-6.

21. Zero DT. In situ caries models. Adv Dent Res 1995;9(3):214-30; discussion 231-4.

22. Fonseca RB, Haiter-Neto F, Fernandes-Neto AJ, Barbosa GA, Soares CJ. Radiodensity of enamel and dentin of human, bovine and swine teeth. Arch Oral Biol 2004;49(11):919-22.

23. Moriwaki Y, Kani T, Kozatani T, Tsutsumi S, Shimode N, Yamaga R. The crystallinity change of bovine enamel during maturation. Jpn J Dent Mat 1968;9:78-85.

24. Yu KC, Chang R. Adhesive restorative dental materials II. In: Approaches to achieve adhesion: National Institute of Dental Research: U.S. Department of Health, Education and Welfare; 1966. p. 103-131.

25. Causton BE. Improved bonding of composite restorative to dentine. A study in vitro of the use of a commercial halogenated phosphate ester. Br Dent J 1984;156(3):93-5.

26. McCabe JF, Rusby S. Dentine bonding agents--characteristic bond strength as a function of dentine depth. J Dent 1992;20(4):225-30.

27. Suzuki T, Finger WJ. Dentin adhesives: site of dentin vs. bonding of composite resins. Dent Mater 1988;4(6):379-83.

28. Oilo G, Olsson S. Tensile bond strength of dentin adhesives: a comparison of materials and methods. Dent Mater 1990;6(2):138-44.

29. Lopes MB, Sinhoreti MA, Correr Sobrinho L, Consani S. Comparative study of the dental substrate used in shear bond strength tests. Pesqui Odontol Bras 2003;17(2):171-5.

FIGURES

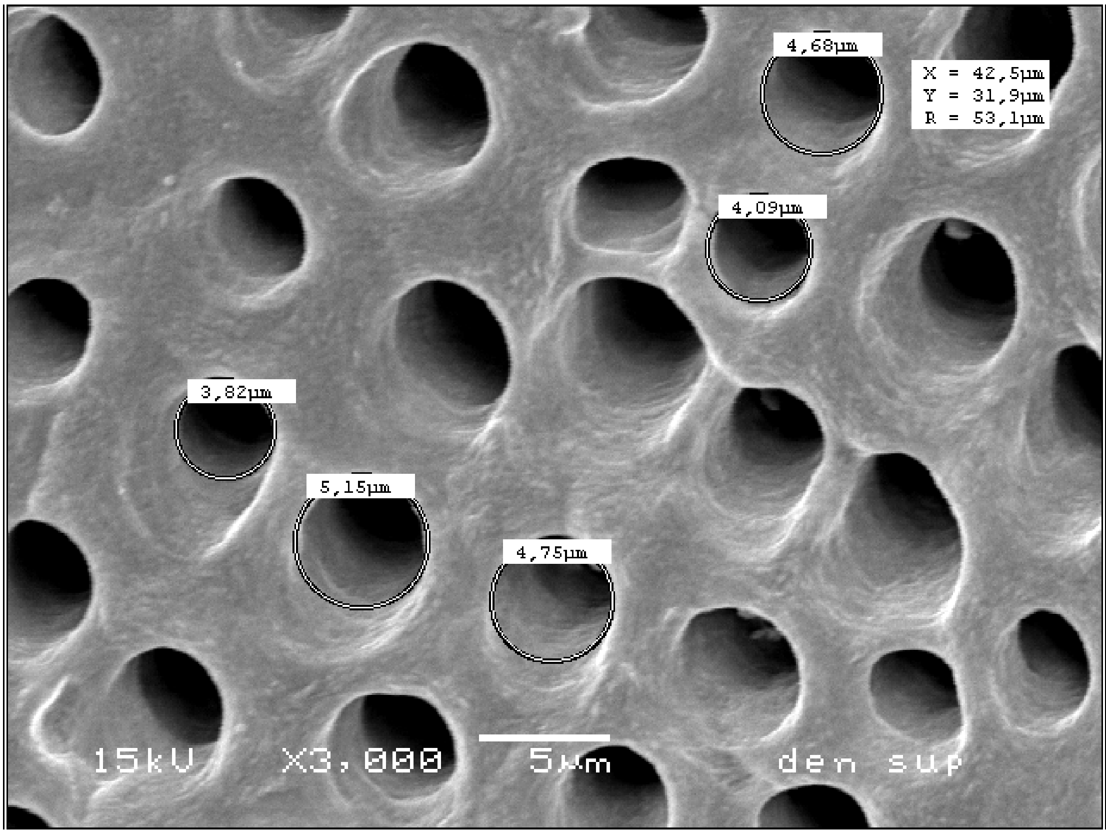


Figure 1 – Dentinal tubules and total area measured using SEM software in human dentine

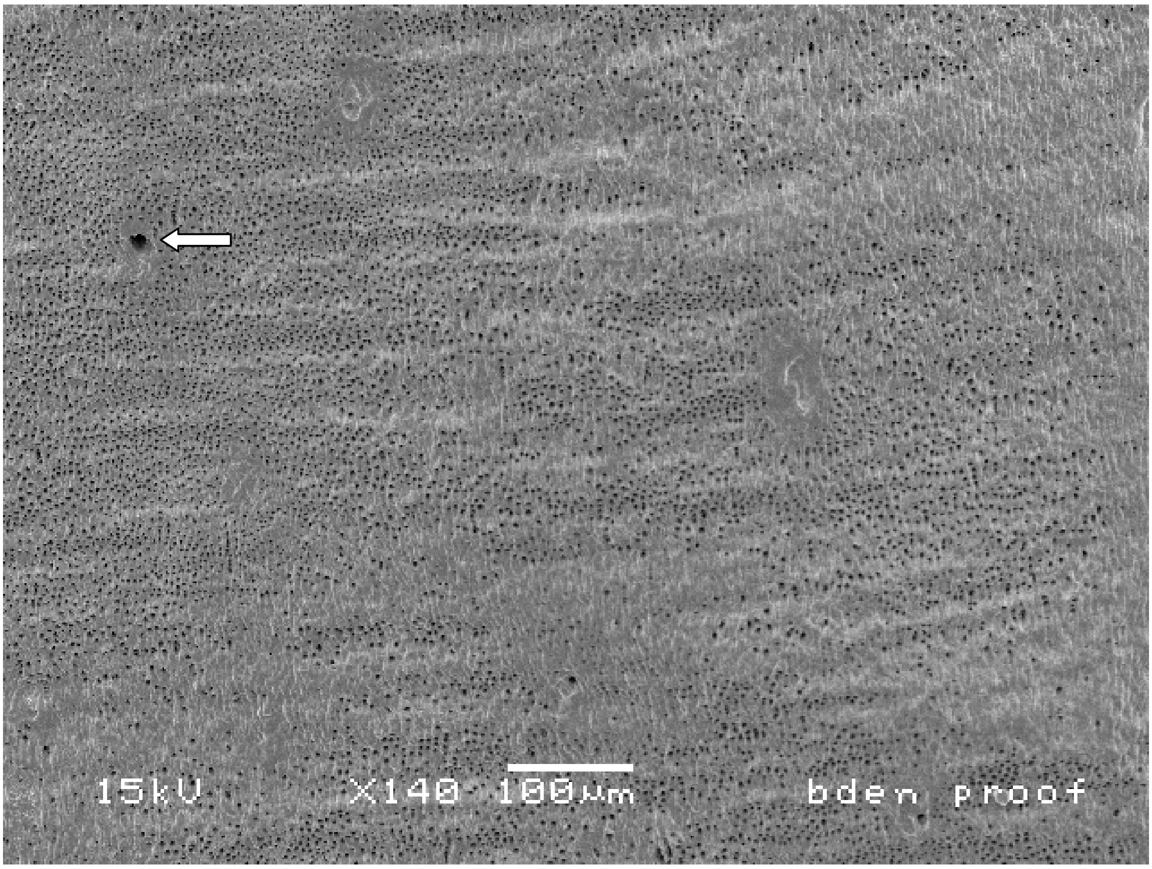


Figure 2 – Conglomerate of dentinal tubules in bovine tooth (X140)

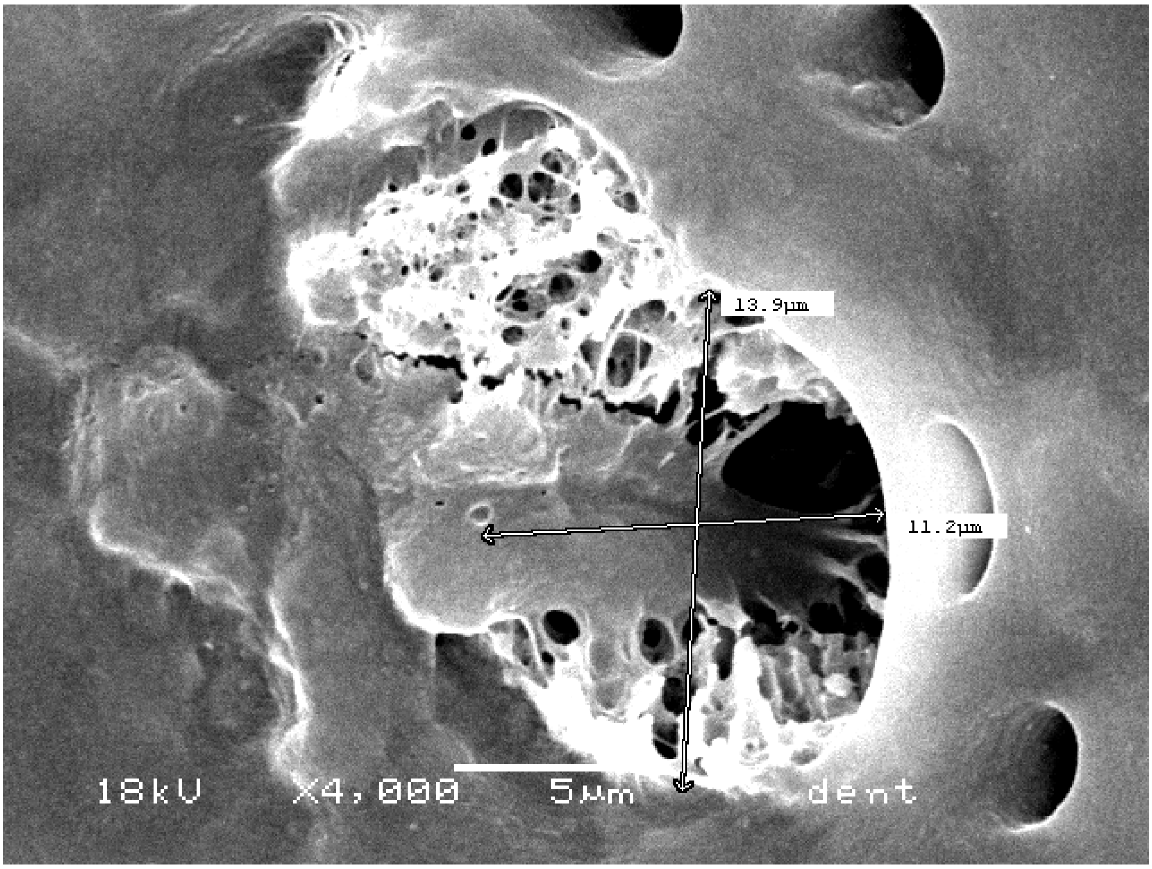


Figure 3 – Conglomerate of dentinal tubules in bovine tooth (X4000)

TABLES

Table 1 – Comparison of tubular diameter (µm)

Depth \ Substrate	Human	Bovine
Superficial	2.42 (0.56) bA	4.21 (0.44) aB
Middle	2.94 (0.38) aA	3.98 (0.26) aB
Deep	2.99 (0.44) aA	3.14 (0.41) bA

Means followed by the same small letters in each column and by the same capital letter in each row are not significant difference (p>0.05). Figures in parentheses are standard deviations.

Table 2 – Comparison of tubular density (tubules/mm^2)

Depth \ Substrate	Human	Bovine
Superficial	15,385 (5,309) aA	11,530 (2875) aB
Middle	21,006 (7,473) bA	15,414 (5289) bB
Deep	30,595 (12,247) cA	20,948 (4558) cB

Means followed by the same small letter in each column and by the same capital letter in each row are not significantly different (p>0.05). Figures in parentheses are standard deviations

4. CAPÍTULO 3

Comparison of two techniques to determine microleakage for different substrates

Artigo a ser enviado para Journal of Dentistry

ABSTRACT

Objective: To compare two techniques (confocal and optical microscopies) for determining microleakage in human and bovine teeth for adhesive bond systems.

Methods: 20 human teeth and 20 bovine teeth had cavities (3mmØX1.5mm) made on the buccal surface. The teeth were divided into 4 groups (n=10) according to the substrate and adhesive (Clearfil SE Bond (Kuraray, Japan) or Scotchbond 1 (3M, USA)). Composite (Wave-SDI, Australia) was applied in two increments, each cured for 30s. The specimens were stored in 100% relative humidity at 37°C for 24h and submitted to 1000 thermal cycles (5-55^{o}C 1min dwell), followed by immersion in aqueous rhodamine 0.6% for 48h. They were rinsed and sectioned at the centre of the restoration. Microleakage was measured and a score was allocated using the scale: 0–none, 1–up to enamel junction, 2–up to pulp wall, 3-in pulp wall, 4–beneath pulp wall. Penetration was submitted to ANOVA and Tukey test (5%) and the scores were submitted to a Kruskal-Wallis and Dunn's multiple comparison test (5%).

Results: When bovine teeth were used, Scotchbond (confocal-87.76%d/optical-68.23%c) showed statistically higher penetration mean than Clearfil (confocal-66.22%c/optical-47.25%b) inside the same microscopy group. When human teeth were used no difference was found between Scotchbond (confocal-47.35%b /optical-22.82%a) and Clearfil (confocal-36.01%b/12.99%a) inside the same microscopy group. When scores were analyzed, Scotchbond showed no difference to Clearfil, however Clearfil showed statistically less infiltration in bovine teeth than in human teeth when optical microscopy was used.

Conclusions: Confocal microscopy showed higher sensitivity for the stain and clearer leakage limits. Bovine teeth may be not suitable for adhesive microleakage tests.

INTRODUCTION

Nowadays, with the advancement in dentistry, a better understanding of dentine substrate characteristics has provided a significant decrease in marginal leakage of restorations.(Pazinatto *et al*, 2003) However, resin composites still present polymerization shrinkage and different linear coefficients of thermal expansion from the tooth. The linear coefficients of thermal expansion of a material, thermal or occlusal stresses and polymerization contraction have been pointed out as a factor influencing microleakage.(Nelsen *et al*, 1952)

Clinical failure of composite restorations is often the result of an incomplete sealing of the tooth/restoration interface.(Hilton, 2002) The role of composite polymerisation stress as one of the main causes of marginal integrity loss and consequent post-operative occurrences such as hypersensitivity, marginal staining and secondary caries, has been described by several researches.(St Georges *et al.*, 2002;Eick&Welch, 1986)

In order to predict the clinical performance of enamel and dentine bonding systems, usually three different ways of *in vitro* evaluation are performed: morphological observations, microleakage studies and bond strength tests. For studying the efficacy of the marginal seal due to bonding created under clinical conditions, Class V restorations have shown to be useful models, in dye penetration studies.(Ferrari *et al.*, 2000) Microleakage tests, which has been defined as the clinically undetectable passage of fluids and bacteria between a restorative material and the prepared tooth,(Kidd, 1976) are the most frequently used laboratory tests to study the mechanisms that may minimize, or eliminate, the leakage around central restorations.(Pazinatto *et al*, 2003)

To evaluate the adhesive bond strength, plenty of in vitro research has been developed using extracted human teeth, which are, however, more and more difficult because of preventive dentistry advances.(Lopes *et al*, 2003) Therefore, it became necessary to look for an alternative substrate. As mammalian teeth are histologically and morphologically similar(Weichert&Presch, 1975), investigators have turned to teeth from bovine, ovine, equine, or swine to provide quantities of standardized material for their studies. Bovine teeth, because their availability as well as their larger size have been used as substitutes for human substrate.(Abuabara *et al.*, 2004)

When optical microscopy is used to determinate microleakage, the limit between leakage and excess of stain is sometimes very difficult to differentiate, due to the stain spread. Confocal laser scanning microscopy (CLSM) is a non-destructive technique for visualizing subsurface features of tissues, and can be applied to detect fluorescence deep within tissues.(Watson *et al.*, 2000) As it has the property to observe structures beneath the surface, it may have advantages over optical microscopy to establish leakage limits of the specimens.

The hypotheses tested were if the confocal microscopy shows clearer limits of leakage when compared to the optical microscopy and if the bovine tooth present similar pattern of leakage of human teeth.

MATERIALS AND METHODS

Twenty human third molar teeth, extracted for orthodontic reason, and 20 bovine incisor teeth was used. They were stored in 0.5% aqueous chloramine for 2 months at maximum before use. Cavities (3mmØX1.5mm) were made on the buccal surface using a handpiece with a special device to produce standardized cavities. The teeth were divided into 4 groups of 10 according to the substrate and adhesive applied: Clearfil SE Bond (Kuraray, Japan) or Scotchbond 1 (3M, USA) and cured for 20s. Composite (Wave-SDI, Australia) was applied in two increments, each cured for 30s. All materials were applied according to the manufacturers' instructions. The specimens were stored in 100% relative humidity at 37°C for 24h and then submitted to 1000 thermal cycles at 5 ^{o}C and 55^{o}C with a dwell time of 1 minute in each temperature. The specimens were covered with 2 layers of Nail Varnish (Colorama, Brazil), except the composite restoration and 1mm around it. Followed by immersion in aqueous rhodamine 0.6% for 48h. The specimens were rinsed and sectioned in a saw (model 650, South Bay Tech.) at the centre of the restoration. The specimens were polished using alumina paste in decrescent granulation order of 5µm, 3 µm and 1 µm, followed by ultrasonic bath cleaner. Microleakage was measured using optical microscopy (Zeiss) at 25X magnification and confocal microscopy (Zeiss) at 25X magnification using fluorescent mode. Around three photographs were taken from each specimen in order to obtain the full perimeter of the restoration and the leakage was measured using software (UTHSCSA Imagetool for Windows, v.3.0). The total length of the internal margins of the restorations and the leakage length were measured and the percentage of leakage length over total length was obtained for both microscopy. In both microscopy, a score was allocated using the scale: 0–none, 1–up to enamel junction, 2–up to pulp wall, 3-in pulp wall, 4–beneath pulp wall. Penetration was submitted to three-way ANOVA and Tukey test (5%) and the scores were submitted to a Kruskal-Wallis and Dunn's multiple comparison test (5%).

RESULTS

Table 1 – Means of penetration in percentage (SD) and median of scores

		Confocal		Optical	
		Clearfil	Scotchbond	Clearfil	Scotchbond
Human	mean(SD)	36.01%(15.55)b	47.35%(9.21)b	12.99%(9.75)a	22.82%(13.33)a
	median	2AB	3B	1.5A	3AB
Bovine	mean(SD)	66.22%(6.02)c	87.76%(8.14)d	47.25%(13.53)b	68.23%(14.14)c
	median	3B	3B	3B	3B

Means followed by different small letters and median followed by different capital letters means statistical difference

When bovine teeth were used, Scotchbond showed statistically higher mean penetration than Clearfil. When human teeth were used no difference was found. When scores were analyzed, Scotchbond showed no difference to Clearfil, however Clearfil showed statistically less infiltration in bovine teeth than in human teeth when optical microscopy was used.

Confocal microscopy always showed higher percentage of leakage with statistical difference from optical microscopy.

DISCUSSION

A desirable property of dentine bonding systems is that they seal the resin-dentine interface, preventing exposure of the pulp-dentine complex to bacteria and their toxins.(Perdigao *et al.*, 1996) A variety of factors can affect the bond formation, such as the density and orientation of dentine tubules,(Harnirattisai *et al.*, 1992) the depth of the cavity,(Pashley *et al.*, 1993) the type of dentine suface,(Mjor&Nordahl, 1996) the presence of sclerotic dentine,(Yoshiyama *et al.*, 1996) and the different *in vivo* conditions. In order to simulate *in vivo* conditions, thermocycling bath is commonly used, based on the fact it simulates the temperatures changes in the mouth.(Celiberti&Lussi, 2005) The difference of linear coefficient of thermal expansion between the substrate and the restorative materials has been suggested as an important factor that influences microleakage.(Nelsen *et al*, 1952) A greater difference in the linear coefficient of thermal expansion between tooth and restorative material will alter the dimensions of the adhesive interface with temperature change.(Trowbridge, 1987)

The hypothesis that confocal microscopy shows clearer limits of leakage when compared to optical microscopy was accepted. It can be noticed on figures 1 and 2, which show the same specimen observed in both microscopes. The clearer limits for confocal microscopy is due to the focus of its lens that occur some microns beneath the observed surface, eliminating the stain spread caused by the sectioning of the specimen(Mannocci *et al.*, 2005) and also avoid polishing artefacts that overestimate the penetration of the dyes(Watson, 1997). According to Minsky(Minsky, 1988), the confocal principle that the illumination and imaging of one spot in one focal plane at one time are combined creating the elimination of scattered, reflected and fluorescent light from out of the focus planes make possible the subsurface observation.

The confocal microscopy also demonstrated higher means of leakage than optical microscopy. The sensibility of the stain visualization may be higher for confocal microscopy than to optical, resulting in high values of leakage. However, the pattern of the results was similar for both microscopies when comparing human with bovine teeth.

Human teeth must be considered the most appropriate hard tissue substrate for *in vitro* studies from the perspective of clinical relevance(Zero, 1995). However, human teeth are of a highly variable composition due to genetic influences, environmental conditions and age. As for enamel is concerned, bovine enamel has a more uniform composition than human enamel, and thus provides a less variable substrate for research purposes. However bovine enamel is more porous and demineralises faster(Mellberg, 1992;Zero, 1995) than human enamel. Edmunds *et al.*(Edmunds *et al*, 1988) concluded from their work on human teeth that the considerable variability in structure of human surface enamel may result in variation in caries susceptibility between individuals. Furthermore, Fonseca *et al.*(Fonseca *et al*, 2004) found that bovine and swine teeth have lower values of radio density when compared to human teeth. This confirms that the mineral composition of human and bovine teeth is different.

Among the different groups of teeth, incisors are preferred for *in vitro* studies when using bovine teeth due to their size and relatively flat buccal surface.(Mellberg, 1992) When using human teeth, it is difficult to obtain non-carious incisors and therefore molars are preferred.

In this study, the microleakage of human and bovine teeth was compared. Bovine teeth showed high means of leakage with statistical difference when compared to human teeth in all groups. Oesterle *et al*.(Oesterle *et al.*, 1998) verified that the enamel bond in bovine teeth is 21% to 44% weaker then that in human enamel and may explain the greater microleakage values in bovine teeth.(Abuabara *et al*, 2004)

It was also noticed different pattern of results between human and bovine groups. When Scotchbond was used, statistical differences were found when using bovine teeth, however the same did not happened when human teeth were used. When scores were analyzed, Clearfil showed statistical difference when bovine teeth were used, but not when human teeth were used. Reeves *et al*.(Reeves *et al*, 1995) and Abuabara *et al*.(Abuabara *et al*, 2004) found differences in the material rank with different substrates in a microleakage test. The second hypothesis of this study that bovine teeth present similar pattern of leakage of human teeth must be rejected.

CONCLUSIONS

1 - When bovine teeth were used, Scotchbond showed statistically higher mean penetration than Clearfil. When human teeth were used no difference was found.

2 - When scores were analyzed, Scotchbond showed no difference to Clearfil, however Clearfil showed statistically less infiltration in bovine teeth than in human teeth when optical microscopy was used.

3 – Confocal microscopy showed higher sensibility for the stain and clearer leakage limits.

4 – Bovine teeth may be not suitable for adhesive microleakage tests

ACKNOWLEDGMENT

Grateful acknowledgement is made to CAPES for its support in this research - BEX: 3427/04-8.

REFERENCES

1. Pazinatto FB, Campos BB, Costa LC, Atta MT. Effect of the number of thermocycles on microleakage of resin composite restorations. Pesquisa Odontologica Brasileira = Brazilian Oral Research. 2003;17(4):337-41.

2. Nelsen RJ, Wolcott RB, Paffenbarger GC. Fluid exchange at the margins of dental restorations. J Am Dent Assoc. 1952 Mar;44(3):288-95.

3. Hilton TJ. Can modern restorative procedures and materials reliably seal cavities? In vitro investigations. Part 2. Am J Dent. 2002 Aug;15(4):279-89.

4. St Georges AJ, Wilder AD, Jr., Perdigao J, Swift EJ, Jr. Microleakage of Class V composites using different placement and curing techniques: an in vitro study. Am J Dent. 2002 Aug;15(4):244-7.

5. Eick JD, Welch FH. Polymerization shrinkage of posterior composite resins and its possible influence on postoperative sensitivity. Quintessence Int. 1986 Feb;17(2):103-11.

6. Ferrari M, Mason PN, Vichi A, Davidson CL. Role of hybridization on marginal leakage and bond strength. American Journal of Dentistry. 2000;13(6):329-36.

7. Kidd EA. Microleakage: a review. J Dent. 1976 Sep;4(5):199-206.

8. Lopes MB, Sinhoreti MA, Correr Sobrinho L, Consani S. Comparative study of the dental substrate used in shear bond strength tests. Pesqui Odontol Bras. 2003 Apr-Jun;17(2):171-5.

9. Weichert CK, Presch W. Elements of chordate anatomy. New York ; London: McGraw-Hill; 1975.

10. Abuabara A, Santos AJ, Aguiar FH, Lovadino JR. Evaluation of microleakage in human, bovine and swine enamels. Pesquisa Odontologica Brasileira = Brazilian Oral Research. 2004;18(4):312-6.

11. Watson TF, Azzopardi A, Etman M, Cheng PC, Sidhu SK. Confocal and multi-photon microscopy of dental hard tissues and biomaterials. Am J Dent. 2000 Nov;13(Spec No):19D-24D.

12. Perdigao J, Lambrechts P, Van Meerbeek B, Braem M, Yildiz E, Yucel T, et al. The interaction of adhesive systems with human dentin. Am J Dent. 1996 Aug;9(4):167-73.

13. Harnirattisai C, Inokoshi S, Shimada Y, Hosoda H. Interfacial morphology of an adhesive composite resin and etched caries-affected dentin. Oper Dent. 1992 Nov-Dec;17(6):222-8.

14. Pashley EL, Tao L, Matthews WG, Pashley DH. Bond strengths to superficial, intermediate and deep dentin in vivo with four dentin bonding systems. Dent Mater. 1993 Jan;9(1):19-22.

15. Mjor IA, Nordahl I. The density and branching of dentinal tubules in human teeth. Arch Oral Biol. 1996 May;41(5):401-12.

16. Yoshiyama M, Sano H, Ebisu S, Tagami J, Ciucchi B, Carvalho RM, et al. Regional strengths of bonding agents to cervical sclerotic root dentin. J Dent Res. 1996 Jun;75(6):1404-13.

17. Celiberti P, Lussi A. Use of a self-etching adhesive on previously etched intact enamel and its effect on sealant microleakage and tag formation. J Dent. 2005 Feb;33(2):163-71.

18. Trowbridge HO. Model systems for determining biologic effects of microleakage. Oper Dent. 1987 Autumn;12(4):164-72.

19. Mannocci F, Sherriff M, Watson TF, Vallittu PK. Penetration of bonding resins into fibre-reinforced composite posts: a confocal microscopic study. Int Endod J. 2005 Jan;38(1):46-51.

20. Watson TF. Fact and artefact in confocal microscopy. Adv Dent Res. 1997 Nov;11(4):433-

21. Minsky M. Memoir on inventing the confocal scanning microscope. Scanning. 1988;10:128-38.

22. Zero DT. In situ caries models. Adv Dent Res. 1995 Nov;9(3):214-30; discussion 31-4.

23. Mellberg JR. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity in situ. J Dent Res. 1992 Apr;71 Spec No:913-9.

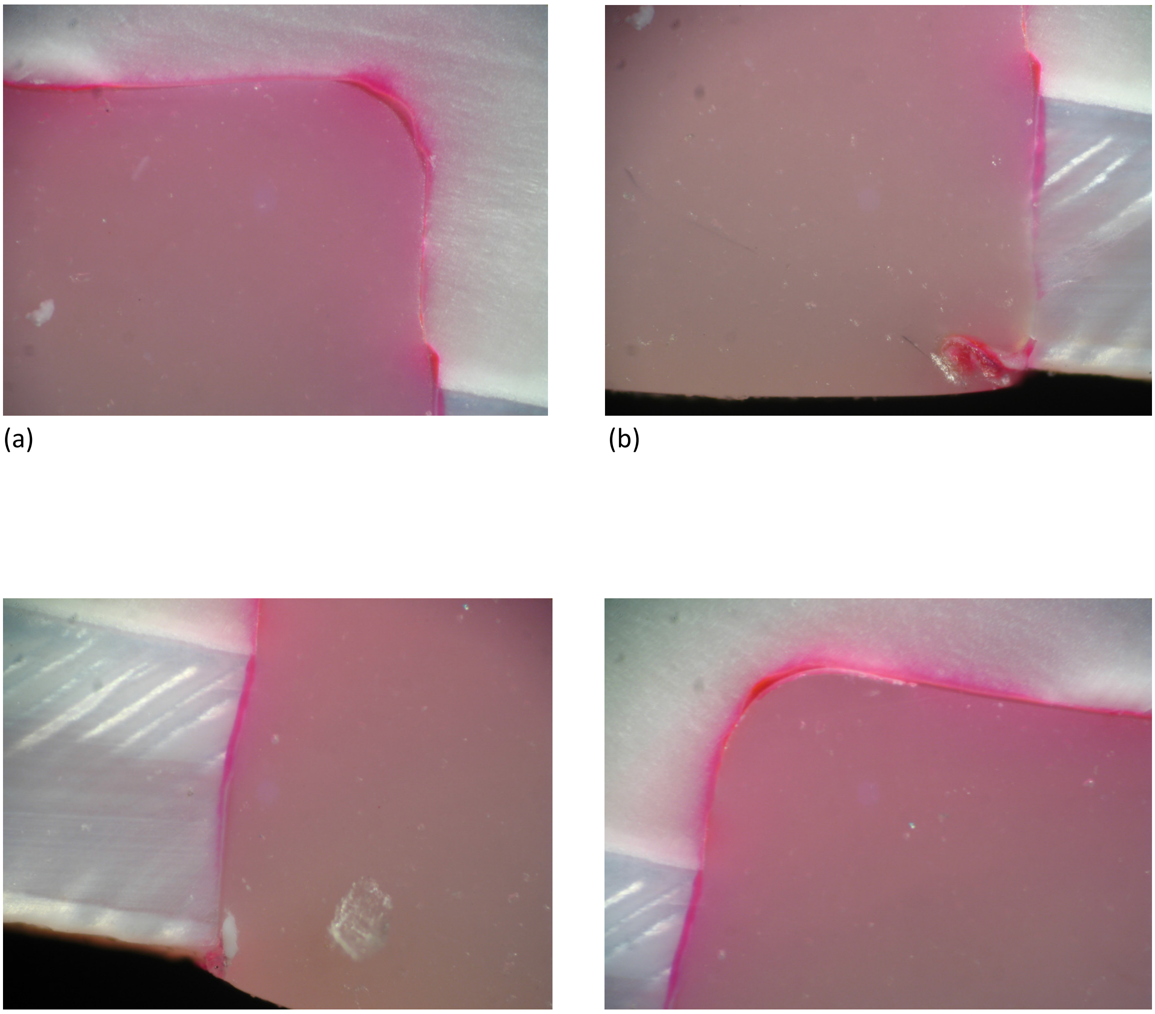
24. Edmunds DH, Whittaker DK, Green RM. Suitability of human, bovine, equine, and ovine tooth enamel for studies of artificial bacterial carious lesions. Caries Res. 1988;22(6):327-36.

25. Fonseca RB, Haiter-Neto F, Fernandes-Neto AJ, Barbosa GA, Soares CJ. Radiodensity of enamel and dentin of human, bovine and swine teeth. Arch Oral Biol. 2004 Nov;49(11):919-22.

26. Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. Am J Orthod Dentofacial Orthop. 1998 Nov;114(5):514-9.

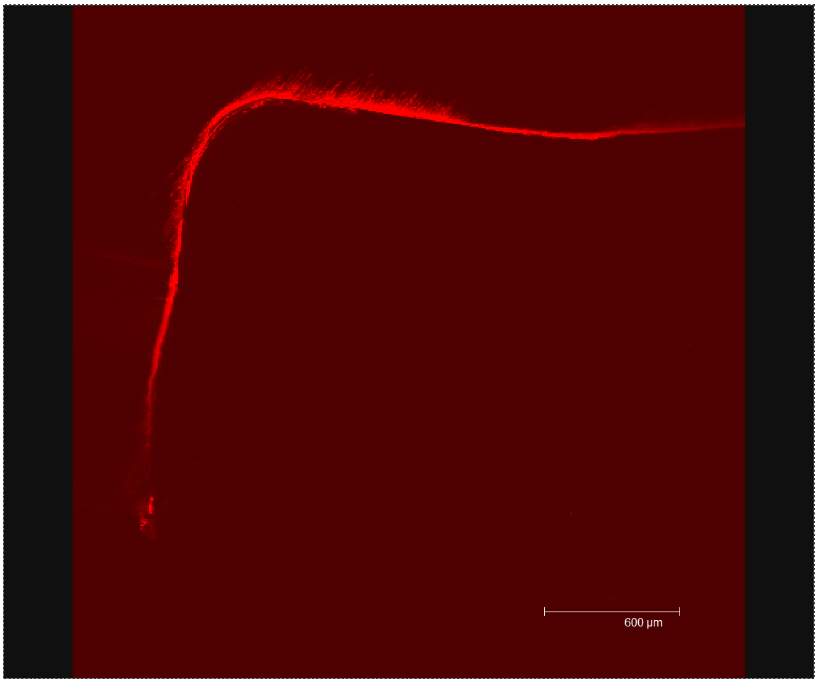
27. Abuabara A, Santos AJ, Aguiar FH, Lovadino JR. Evaluation of microleakage in human, bovine and swine enamels. Pesqui Odontol Bras. 2004 Oct-Dec;18(4):312-6.

28. Reeves GW, Fitchie JG, Hembree JH, Jr., Puckett AD. Microleakage of new dentin bonding systems using human and bovine teeth. Oper Dent. 1995 Nov-Dec;20(6):230-5.

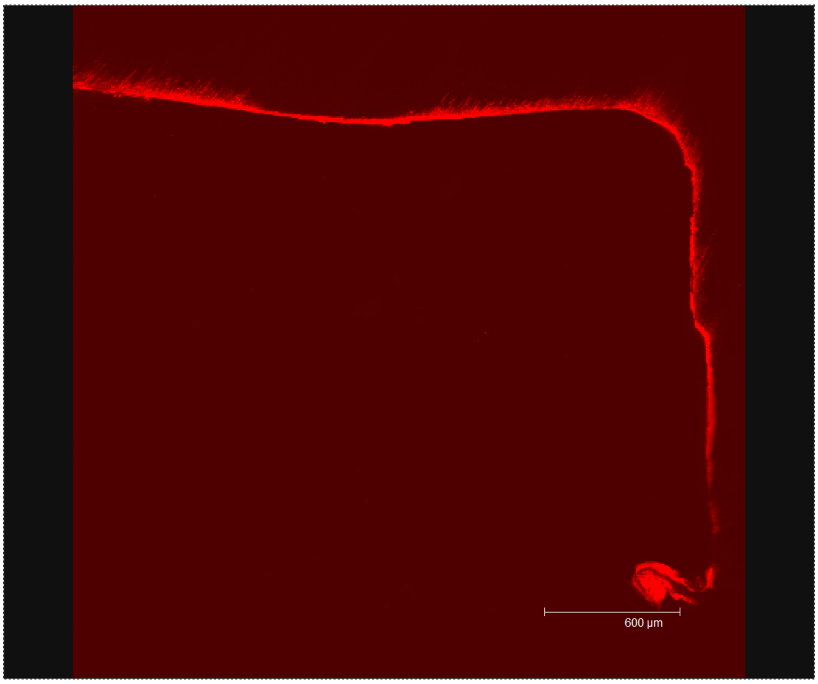


(a) (b) (c) (d)

Figure 1 – Microleakage in bovine tooth using Scotchbond 1 in optical microscopy. Figures 1a,1b,1c,1d shows the full perimeter of restoration.



(a)



(b)

Figure 2 – Microleakage in bovine tooth using Scotchbond 1 in confocal microscopy. Figures 2a,2b shows the full perimeter of restoration.

5. CONCLUSÃO GERAL

1 – A substuituição do dente bovino pelo humano em testes laboratoriais para união parece ser parcialmente válido, porque os valores obtidos nem sempre são correlatos aos dois substratos fato que depende do adesivo utilizado

2 – Quanto ao diâmetro tubular, a dentina bovina superficial e média mostrou diâmetros estatisticamente maiores que a dentina humana superficial e média.

3 - Não houve diferença estatística para o diâmetro tubular entre dentina humana e bovina, na dentina profunda.

4 - A densidade dos túbulos dentinários foi estatisticamente maior em dentina humana que em dentina bovina.

5 - O dente bovino não seria o mais adequado para teste de microinfiltração.

REFERÊNCIAS[2]

Arends J, Schuthof J, Jongebloed WG. Lesion depth and microhardness indentations on artificial white spot lesions. Caries Res. 1980;14(4):190-5.

Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955 Dec;34(6):849-53.

Burrow MF, Sano H, Nakajima M, Harada N, Tagami J. Bond strength to crown and root dentin. Am J Dent. 1996 Oct;9(5):223-9.

Featherstone JD, Mellberg JR. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. Caries Res. 1981;15(1):109-14.

Fusayama T, Nakamura M, Kurosaki N, Iwaku M. Non-pressure adhesion of a new adhesive restorative resin. J Dent Res. 1979 Apr;58(4):1364-70.

Groenhuis RA, Jongebloed WL, ten Bosch JJ. Surface roughness of acid-etched and demineralized bovine enamel measured by a laser speckle method. Caries Res. 1980;14(5):333-40.

Kidd EA. Microleakage: a review. J Dent. 1976 Sep;4(5):199-206.

Marshall GW, Jr. Dentin: microstructure and characterization. Quintessence Int. 1993 Sep;24(9):606-17.

Mellberg JR. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity in situ. J Dent Res. 1992 Apr;71 Spec No:913-9.

Nakamichi I. [Adhesion of various dental restorative materials to human and bovine teeth (author's transl)]. Kokubyo Gakkai Zasshi. 1982 Mar;49(1):31-40.

Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res. 1983 Oct;62(10):1076-81.

Nelsen RJ, Wolcott RB, Paffenbarger GC. Fluid exchange at the margins of dental restorations. J Am Dent Assoc. 1952 Mar;44(3):288-95.

Olsson S, Oilo G, Adamczak E. The structure of dentin surfaces exposed for bond strength measurements. Scand J Dent Res. 1993 Jun;101(3):180-4.

[2] De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

Pazinatto FB, Campos BB, Costa LC, Atta MT. Effect of the number of thermocycles on microleakage of resin composite restorations. Pesquisa Odontologica Brasileira = Brazilian Oral Research. 2003;17(4):337-41.

Pearce EI. A microradiographic and chemical comparison of in vitro systems for the simulation of incipient caries in abraded bovine enamel. J Dent Res. 1983 Sep;62(9):969-74.

Putt MS, Kleber CJ, Muhler JC. A comparison of the polishing properties of human and bovine enamel. J Dent Res. 1980 Jul;59(7):1177.

Reeves GW, Fitchie JG, Hembree JH, Jr., Puckett AD. Microleakage of new dentin bonding systems using human and bovine teeth. Oper Dent. 1995 Nov-Dec;20(6):230-5.

Rueggeberg FA. Substrate for adhesion testing to tooth structure - review of the literature. Dent Mater. 1991 Jan;7(1):2-10.

Schilke R, Lisson JA, Bauss O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol. 2000 May;45(5):355-61.

Schilke R, Bauss O, Lisson JA, Schuckar M, Geurtsen W. Bovine dentin as a substitute for human dentin in shear bond strength measurements. Am J Dent. 1999 Apr;12(2):92-6.

Sydney-Zax M, Mayer I, Deutsch D. Carbonate content in developing human and bovine enamel. J Dent Res. 1991 May;70(5):913-6.

Weichert CK, Presch W. Elements of chordate anatomy. New York ; London: McGraw-Hill; 1975.

ANEXOS

UNICAMP

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

MENTE·MANV·CORDE

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Estudo comparativo do tipo de substrato dental utilizado em testes de resistência de união", sob o protocolo nº **055/2001**, do Pesquisador ***Murilo Baena Lopes***, sob a responsabilidade do Prof. Dr. **Mário Alexandre Coelho Sinhoreti**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 31 de maio de 2001

We certify that the research project with title "Comparative study of two substrates used in bond strength tests", protocol nº **055/2001,** by Researcher ***Murilo Baena Lopes***, responsibility by Prof. Dr. **Mário Alexandre Coelho Sinhoreti**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Resarch at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, May 31 2001

Prof. Dr. Pedro Luiz Rosalen
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador
CEP/FOP/UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Tese de Doutorado intitulada "ANÁLISE ESTRUTURAL E MECÂNICA DE DENTES BOVINOS RELACIONADOS A TESTES DE UNIÃO ADESIVA", não infringem os dispositivos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

Piracicaba, 30 de Janeiro de 2007.

MURILO BAENA LOPES
RG: 28467250-9
Autor(a)

SIMONIDES CONSANI
RG: 2640729
Orientador(a)