ANDERSON TAKEO HARA

EFEITO DE MATERIAIS RESTAURADORES FLUORETADOS NO DESENVOLVIMENTO DE CÁRIE SECUNDÁRIA EM DENTINA RADICULAR

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Doutor em Clínica Odontológica - Área de Dentística

PIRACICABA
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2004

Ficha Catalográfica

Hara, Anderson Takeo.
H212e Efeito de materiais restauradores fluoretados no desenvolvimento de cárie secundária em dentina radicular. / Anderson Takeo Hara. - Piracicaba, SP : [s.n.], 2004.
xiv, 110p. : il.

Orientadores : Prof[a] Dr[a] Mônica Campos Serra, Prof. Dr. Jaime Aparecido Cury, Prof. Dr. Antonio Luiz Rodrigues Jr.
Tese (Doutorado) - Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Cáries dentárias. 2. Materiais dentários. 3. Dentes - Tratamento. 4. Dentifrício. 5. Dentina. 6. Odontologia (Restauração). I. Serra, Mônica Campos. II. Cury, Jaime Aparecido. III. Rodrigues Jr., Antonio Luiz. IV. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. V. Título.

Ficha catalográfica elaborada pela Bibliotecária Marilene Girello CRB/8–6159, da Biblioteca da Faculdade de Odontologia de Piracicaba - UNICAMP.

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 16 DE AGOSTO DE 2004, CONSIDEROU O CANDIDATO ANDERSON TAKEO HARA APROVADO.

1. PROFA. DRA. MÔNICA CAMPOS SERRA_____
2. PROFA. DRA. MARIA FIDELA DE LIMA NAVARRO_____
3. PROF. DR. CARLOS FRANCCI_____
4. PROFA. DRA. CINTHIA PEREIRA MACHADO TABCHOURY_____
5. PROF. DR. MARCELO GIANNINI_____

DEDICATÓRIA

Dedico este trabalho aos meus orientadores: Profs. Drs. **MÔNICA CAMPOS SERRA** e **JAIME A. CURY** pela inestimável contribuição ao meu desenvolvimento científico, acadêmico e pessoal.

E a meus pais: **TAKEHIRO** e **MIE HARA**, irmãs: **MÔNICA** e **DÉBORA**, esposa: **FERNANDA** e filho: **VÍTOR** pelo constante apoio e suporte.

AGRADECIMENTOS

À FOP/UNICAMP, na pessoa do diretor Prof. Dr. Thales Rocha de Mattos Filho, ao Curso de Pós-graduação da FOP/UNICAMP, na pessoa do coordenador Prof. Dr. Pedro Luiz Rosalen e ao Programa de Pós-graduação em Clínica Odontológica da FOP/UNICAMP, na pessoa do coordenador Prof. Dr. Roger William Fernandes Moreira.

À FAPESP, pelo apoio financeiro concedido durante o desenvolvimento deste e de outros trabalhos, e à CAPES, por possibilitar a realização de estágio de doutorado sanduíche no exterior.

Ao *Oral Health Research Institute* (OHRI)/*Indiana University*, por me receber para a realização do estágio de doutorado sanduíche e a todos os Profs. e funcionários deste Instituto, em especial ao Prof. Domenick T. Zero, pela recepção, orientação e ajuda.

À colega Cecilia Pedroso Turssi, pela parceria desde os tempos de mestrado e sobretudo pela ajuda e companheirismo nas dificuldades e desafios vencidos durante o curso de doutorado. Aos colegas Patrícia M. Freitas, Érica C. N. Teixeira, Márcio de Menezes e Silvia J. Domiciano, pela amizade e auxílio na condução de novas pesquisas.

À Profa. Dra. Cinthia P. M. Tabchoury, aos técnicos Mariza de Jesus C. Soares, José Alfredo da Silva e Waldomiro Vieira Filho e a todos os colegas da Área de Bioquímica da FOP/UNICAMP, especialmente Adriana Franco Paes Leme, Carolina P. Aires, Celso da Silva Queiroz, Lívia M. Tenuta, Mitsue F. Hayacibara e Nilza C. L. A. V. Gonçalves.

Aos Profs. e funcionários da Área de Dentística da FOP/UNICAMP, em especial aos Profs. Drs. Marcelo Giannini e Luís Alexandre Maffei Sartini Paulillo.

Ao Prof. Dr. Antonio Luiz Rodrigues Jr., pelos valiosos ensinamentos e conselhos em estatística experimental.

À Profa. Dra. Altair A. Del Bel Cury, pelas orientações relacionadas à bolsa de doutorado sanduíche (PDEE/CAPES).

Aos Profs. Drs. Décio Barbin, Maria Cristina Stolf Nogueira e Elliot W. Kitajima, da Escola Superior de Agricultura Luiz de Queiroz (ESALQ)/USP, pelos ensinamentos na área de Bioestatística e de Microscopia Eletrônica; aos Profs. Drs. Benedito de Moraes Purquerio e João M.D.A. Rollo, da Escola de Engenharia de São Carlos (EESC)/USP, por possibilitarem a realização das disciplinas de Tribologia e Biomateriais; aos professores e aos funcionários do Departamento de Odontologia Restauradora da FORP/USP, por sempre me acolherem durante as visitas à minha orientadora.

Aos Profs. Drs. Jaime A. Cury, Luís Alexandre M. S. Paulillo e Regina Guenka Palma Dibb, membros da banca do exame de qualificação, pela contribuição para o aperfeiçoamento deste trabalho.

Aos funcionários da FOP/UNICAMP, em especial aos da Secretaria de Pós-graduação e Biblioteca pela orientação, constante ajuda e amizade.

Por fim, agradeço a todos que, de uma maneira ou de outra, me ajudaram na concretização deste trabalho.

SUMÁRIO

RESUMO

Materiais restauradores fluoretados têm demonstrado capacidade de inibir a progressão da cárie secundária em dentina radicular *in vitro*. Entretanto, este potencial cariostático ainda necessita ser avaliado em determinados materiais restauradores e testado quanto à sua extensão a partir da margem da restauração e à sua efetividade *in vivo*. Este trabalho, apresentado na forma de 4 artigos, teve por objetivos (1) avaliar o efeito cariostático *in vitro* de sistemas adesivos contendo fluoretos (SAF) em dentina radicular, (2) determinar a extensão do efeito cariostático *in vitro* de restaurações realizadas com cimento de ionômero de vidro (CIV), ionômero de vidro modificado por resina (IVMR), resina composta modificada por poliácidos (RCMP) e resina composta fluoretada (RCF) em dentina radicular, (3) desenvolver um modelo *in situ* para o estudo da cárie radicular e (4) testar se o CIV é importante na inibição do desenvolvimento de cárie secundária radicular *in situ* quando um dentifrício fluoretado é constantemente utilizado. Os SAFs não demonstraram efeito cariostático. O CIV e o IVMR demonstraram efeito cariostático em dentina radicular até 0,3 e 0,15 mm da margem das restaurações, respectivamente, contrastando com a ausência de efeitos da RCMP e a RCF. O modelo *in situ* proposto demonstrou ser adequado para o estudo da progressão e inibição da cárie radicular. Nenhum efeito cariostático adicional pôde ser atribuído ao CIV ao se utilizar dentifrício fluoretado no modelo de cárie radicular *in situ*. Conclui-se que o efeito cariostático, assim como sua extensão em dentina radicular, é dependente do material restaurador e apresenta relevância questionável em condições *in situ*, ao se utilizar dentifrício fluoretado freqüentemente.

ABSTRACT

In vitro studies have shown that fluoride containing restorative materials can inhibit root dentin secondary caries. However, questions still arise on the potential of some restorative materials, on the extension of the cariostatic effect from the restoration margins and on the *in vivo* ability of such materials to prevent secondary root caries. This thesis, comprised by 4 manuscripts, was designed (1) to test the cariostatic effect of fluoride containing adhesive systems (FAS) *in vitro*, (2) to determine the extension of the cariostatic effect of a glass-ionomer cement (GIC), a resin-modified glass-ionomer (RMGI), a polyacid-modified composite resin (PMCR) and a fluoridated composite resin (FCR) on root dentin *in vitro*, (3) to develop an *in situ* model for the study of root caries and (4) to assess whether the GIC is important or not to prevent secondary root caries *in situ* when fluoride dentifrice is frequently used. The FASs tested showed no evident cariostatic effect. The GIC and the RMGI showed cariostatic effect up to 0.3 and 0.15 mm from the margin of the restoration, respectively, contrasting to the lack of effect of PMCR and FCR. The *in situ* model tested was proven to be adequate to the study of either root caries progression or root caries inhibition. No additional cariostatic effect could be attributed to the GIC when fluoride dentifrice was used *in situ*. It could be concluded that the cariostatic potential as well as the extension of this effect on root dentin is dependent of the fluoride containing restorative material and may not be relevant in *in situ* conditions when fluoride dentifrice is frequently used.

1. INTRODUÇÃO GERAL

Cárie radicular secundária define-se como cárie primária adjacente a restaurações localizadas em superfície radicular (Thylstrup, 1998; Mjör & Toffenetti, 2000; Kidd, 2001). O seu desenvolvimento ocorre devido à metabolização de açúcares fermentáveis pela microbiota do biofilme dental, produzindo ácidos orgânicos capazes de desmineralizar a dentina (Featherstone, 1994; Wefel, 1994; Fejerskov & Nyvad, 1996). Tem-se, assim, um desequilíbrio na dinâmica entre perda e ganho mineral da dentina em relação ao microambiente bucal, com aumento da porosidade dentinária e conseqüente exposição da matriz orgânica (Wefel, 1994). Na persistência do desequilíbrio, pode ocorrer a degradação da matriz orgânica por enzimas bacterianas seguida por novos episódios de desmineralização (Featherstone, 1994), favorecendo a progressão da lesão de cárie secundária. Entretanto, em fases iniciais, o equilíbrio mineral pode ser re-estabelecido através do adequado controle de placa dental e dieta e também pelo uso de fluoretos (Nyvad & Fejerskov, 1986).

Embora a utilização de fluoretos possa ocorrer através de meios coletivos, como água de abastecimento ou sal de cozinha, individuais, como dentifrício ou suplementos, e profissionais, como solução, gel ou verniz, o material restaurador deve ser considerado um meio de destaque para a prevenção da cárie secundária, pois se encontra adjacente ao sítio de desenvolvimento da lesão (Erickson, 1994; Burgess, 1995). Assim, o potencial cariostático de materiais restauradores que liberam fluoretos tem sido comprovado, principalmente em estudos *in vitro*, destacando-se o cimento de ionômero de vidro convencional (CIV) e o ionômero de vidro modificado por resina (IVMR), em detrimento da resina composta modificada por poliácidos (RCMP) e da resina composta

fluoretada (RCF) (Dionysopoulos *et al*., 1998; Millar *et al*., 1998; Torii *et al*., 2001; Hara *et al*., 2002).

Outros materiais, tais como os sistemas adesivos fluoretados (SAF), também têm demonstrado capacidade de liberar íons flúor (McCabe *et al*., 2002) e diferenciam-se dos demais materiais restauradores devido a sua capacidade de penetrar na dentina, podendo constituir uma fonte de fluoretos mais efetiva (Ferracane *et al*., 1998; Han *et al*., 2001; 2002). Entretanto, não se sabe se a concentração de fluoretos liberada seria capaz de exercer algum benefício para a dentina, quer seja na inibição da desmineralização ou na ativação da remineralização da dentina. Tais questionamentos fundamentaram a condução de um estudo avaliando a concentração de fluoretos e o potencial cariostático de sistemas adesivos em dentina radicular [**Capítulo 1**].

A maior prevalência de cárie secundária em superfícies proximais (Schüpbach *et al*., 1992) e próximas às margens gengivais (Lynch & Beighton, 1994), assim como a relativamente maior susceptibilidade à desmineralização da dentina em relação ao esmalte (Wefel, 1994), contribui para que materiais restauradores com propriedades cariostáticas sejam de especial interesse para cavidades localizadas em superfícies radiculares. Embora estudos laboratoriais demonstrem que fluoretos liberados por esses materiais interfiram no desenvolvimento da cárie nas adjacências da restauração (Tam *et al*., 1997; Creanor *et al*., 1998; Dionysopoulos *et al*., 1998; Millar *et al*., 1998; Pereira *et al*., 1998a; Hara *et al*., 2000; Torii *et al*., 2001; Hara *et al*., 2002), não se sabe ao certo qual a extensão desse efeito. Para avaliar essa capacidade dos materiais restauradores fluoretados, um estudo *in vitro* foi delineado e conduzido [**Capítulo 2**].

Embora o efeito cariostático de materiais restauradores fluoretados tenha sido demonstrado por estudos *in vitro*, avaliações *in vivo* têm falhado em corroborar tais

resultados (Randall & Wilson, 1999; Papagiannoulis *et al*., 2002). Esta contradição pode estar na grande diversidade de fatores capazes de influenciar a variável de resposta em questão - potencial cariostático - sob condições clínicas. Assim, modelos experimentais de cárie *in situ*, que atuam como uma "ponte" entre situações clínicas não controladas e situações laboratoriais altamente controladas (Zero, 1995), podem ser uma alternativa para a análise do efeito cariostático de materiais restauradores. Esses modelos são capazes de simular condições intrabucais de desafio cariogênico, permitindo a formação de lesões de cárie secundária em esmalte semelhantes às lesões naturais (Benelli *et al*., 1993). Um estudo foi realizado com o objetivo de se propor e testar um modelo de cárie radicular *in situ* [**Capítulo 3**].

A utilização do modelo desenvolvido permitiu avaliar, de maneira próxima à realidade, se o uso contínuo de outras fontes de íons flúor comuns ao ambiente bucal e de grande abrangência, tais como o dentifrício, pode mascarar o efeito cariostático proporcionado pelo material restaurador (Donly & Kerber, 1999). Essas fontes de fluoretos podem atuar diretamente, interferindo nos processos físico-químicos do desenvolvimento da cárie secundária, ou indiretamente, disponibilizando íons flúor a reservatórios em potencial, tais como tecidos moles, saliva e superfície dental - nas formas de fluoreto de cálcio - e biofilme bacteriano (Duckworth & Morgan, 1991; Shellis & Duckworth, 1994). A liberação de íons flúor desses reservatórios poderia ocorrer como conseqüência de ataques cariogênicos, favorecendo a prevenção e paralisação da lesão de cárie. A hipótese de que materiais restauradores fluoretados podem não ser importantes na prevenção ou paralisação da cárie radicular secundária *in situ*, devido à presença de outras fontes de fluoretos tais como o dentifrício fluoretado, foi sugerida e testada [**Capítulo 4**].

2. PROPOSIÇÃO

Esta tese, apresentada na forma de 4 capítulos - conforme deliberação CCPG 001/98[*] (Anexo 1) - teve como objetivo geral avaliar o efeito de materiais restauradores na prevenção da cárie secundária em dentina radicular. Os seguintes aspectos foram focados:

1. efeito cariostático de sistemas adesivos fluoretados *in vitro*,
2. extensão do efeito cariostático de materiais restauradores fluoretados *in vitro*,
3. proposição e teste de um modelo *in situ* para formação de cárie radicular,
4. efeito cariostático do cimento de ionômero de vidro *in situ*, sob a influência de um dentifrício fluoretado.

[*] Deliberação que dispõe a respeito do formato das teses de mestrado e doutorado aprovadas pela UNICAMP.

3.CAPÍTULOS

3.1. CAPÍTULO 1

SUBMETIDO PARA PUBLICAÇÃO / EUROPEAN JOURNAL OF ORAL SCIENCES (ANEXO 2).

FLUORIDE RELEASE AND SECONDARY CARIES INHIBITION BY ADHESIVE SYSTEMS ON ROOT DENTINE

AUTHORS

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RUNNING TITLE

Fluoride release and cariostatic effect of adhesives

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ABSTRACT

This study tested the fluoride releasing rate and the root caries inhibitory effect of dental adhesives. In phase 1, the fluoride released from samples (n=5) of adhesives: A-Optibond Solo, B-One-up Bond F, C-Prime & Bond NT, D-Tenure Quick and also of the controls: [+]-Glass-ionomer and [-]-Non-fluoride releasing adhesive was daily quantified during a pH-cycling, simulating caries phenomena. In phase 2, restorations were made in bovine root dentine slabs (n=16) with the same adhesives associated with a non-fluoridated composite. Control [+] restorations were entirely made with glass-ionomer. Specimens were thermocycled and submitted to the pH-cycling regimen. Demineralisation areas and the presence of wall-lesion (WL) and inhibition zone (IZ) were determined by polarizing light microscopy in dentine adjacent to the restoration. Control [+] released the highest amount of fluoride followed by A, B and C, all of them differing from each other. No detectable amount of fluoride was released by D and [-]. Lower demineralisation areas were found to [+], whereas groups A-D and [-] did not differ from each other. No WL was detected and higher percentages of IZ were recorded to [+] and A. Although some dental adhesives were able to release fluoride, they could not inhibit secondary caries development.

KEYWORDS

Root caries, dental adhesives, cariostatic effect, fluoride releasing

INTRODUCTION

The development of adhesive restorative materials has allowed the introduction of conservative concepts and techniques to restore root surfaces. Consequently, extensive cavity preparations designed to provide mechanical resistance and retention to the restoration has been gradually changed by minimally invasive procedures, intending to keep the integrity of the teeth. Contrasting to this growing conservative approach, a well-known restoration-related problem still remains, as frequently showed in clinical citations of secondary caries (1).

To prevent the occurrence of this pathology, it is essential to consider its causative and protective factors (1,2). Fluoride has shown a cariostatic effect on root dentine (3), probably by inhibiting the root dentine demineralisation and by enhancing its remineralisation as well (4). Since secondary caries may be defined as primary caries around the restorations (1,5), it has been hypothesized that fluoridated restorative materials could be a source of fluoride to effectively prevent the lesion development (6). Among fluoride-containing restorative materials, dental adhesives seem to be especially attractive, since they not only closely contact but also may partially diffuse into dentine margins of restoration (7,8,9). As a result, fluoride might be present in the specific site of secondary caries, potentially reducing its development.

The capacity of dental adhesives to release fluoride has been showed (7,8,9,10), however it is not known either the fluoride release in conditions mimicking caries development (11) or the effect of this releasing on caries prevention. This study was carried out to evaluate the fluoride releasing rate and also the secondary root caries inhibition of fluoride containing dental adhesives.

MATERIALS AND METHODS

Experimental design

This *in vitro* study was conducted in 2 phases. In phase 1, the capacity of dental adhesives to release fluoride was daily tested, during a cariogenic challenge. In phase 2, the cariostatic effect of the same adhesives on root dentine was tested. The experimental designs of both phases are described in Table 1, as well as the materials in Table 2.

PHASE 1 - FLUORIDE RELEASE

Specimens preparation

Five disk-shaped specimens (10.0 mm in diameter and 0.5 mm in thickness) were randomly prepared from each material, according to an experimental block design. The adhesives were dropped into individual teflon matrices, covered with a polyester strip and a glass plate, and light cured for 1 min. Glass-ionomer cement (control [+]) was mixed according to manufacturer's instruction, placed on the matrix, pressed for 1 min with a glass-plate under a load of 500 g and let to cure for 7 min. The specimens were removed from the matrices and excesses of the material were carefully removed with a surgical blade. A small hole (1 mm in diameter) was done on each specimen to allow its suspension with orthodontic steel wire. Specimens were individually stored in tubes with 100% of relative humidity, at 37ºC $\pm$ 0.1.

Fluoride releasing analysis

The specimens were submitted to an 8-day pH-cycling regimen simulating caries development (11). Each day or cycle consisted in the individual immersion of the specimen in demineralising (1.4 mM Ca, 0.9 mM P, 0.05 M acetate buffer, pH 5.0, 2 mL/specimen) and remineralising solutions (1.5 mM Ca, 0.9 mM P, 0.1 M Tris buffer, pH

7.0, 2 mL/specimen) for, 8 and 16 h, respectively. The solutions were daily renewed and 1 mL of each was collected for fluoride releasing analysis. TISAB III (total ionic strength adjustment buffer, Orion, no. 940911, Boston, MA, USA) was added to the collected samples in the proportion of 1 : 10. Quantification of fluoride in the solution was done with an ion selective electrode (96-09) connected to an ion analyser (Orion EA-940), which was previously calibrated with a series of 8 standard solutions (from 0.03 to 10.0 μg F/mL), in triplicates. The daily fluoride released by each specimen was computed by the sum of the amounts released in de and remineralisation solutions. The cumulative release was computed by the sum of the fluoride released during the 8 testing days.

PHASE 2 - SECONDARY CARIES INHIBITION EVALUATION

Specimens preparation

Root dentine slabs (5 x 5 mm and 3 mm thick) were obtained from bovine incisor teeth, previously stored in thymol 0.1 %. Their external surfaces were flattened in a polishing machine, with # 600, 1200 grit Al_2O_3 abrasive papers. Box-shaped cavities (3.3 x 1.5 mm, 1 mm deep) were prepared on the centre of the slab, with diamond burs (# 2096, KG Sorensen, Barueri, SP, Brazil) mounted in a cavity preparation machine (Marcelo Nucci ME, São Carlos, SP, Brazil), at high-speed rotation and under water/air spray refrigeration.

Cavities of 96 slabs were randomly restored with the six dental materials tested (n = 16), according to an experimental block design. Acid etching was done - when requested - and dental adhesives were applied and light cured following the manufacturers' instructions. A non-fluoridated composite-resin (Filtek Z250, 3M Dental Products, St Paul, MN, USA) was inserted on the cavity in one increment, pressed with a polyester strip and a glass plate for 1 min, under a load of 500 g. The restorative material was light cured for 30 s. On the control [+] group, the glass-ionomer cement was mixed

following the manufacturer's instruction, inserted in the cavity with a syringe (Centrix, Centrix Inc., Shelton, CT, USA), covered with a polyester trip and a glass plate, pressed with a load of 500g and let cure for 7 min. The surface of the glass-ionomer restoration was protected with a nail varnish. Specimens were individually stored at 100 % of relative humidity, at 37º C ± 0.1. After 24 h, the excesses of restorative materials were removed with # 1200 grit Al_2O_3 abrasive papers under water refrigeration and the surface was subsequently polished with abrasive papers and 1 μm diamond suspension on cloths.

Thermalcycling and chemical caries regimen

Specimens, separated according to each group, were submitted to thermal stress in a thermalcycling machine (MSCT-3, Marcelo Nucci ME, São Carlos, SP, Brazil), by the immersion in baths of 5º C ± 1 and 55º C ± 1, for 1 min each. The dwell time between baths was 15s. A total of 1,000 cycles were performed.

Artificial caries challenge was provided to the specimens by the 8-days chemical regimen previously described in phase 1. The contents and volumes of the demineralising and remineralising solutions as well as the design of the artificial root caries cycling regimen were previously defined in preliminary tests. Each day or cycle consisted in the individual immersion of the specimen in demineralising (3.12 mL/mm^2) and remineralising solutions (1.56 mL/mm^2) for, 8 and 16 h, respectively. Between the immersions and at the end of the cycling, the specimens were rinsed with distilled and deionised water and dried.

Secondary caries inhibition evaluation

Specimens were cut vertically through the centre of the restoration, with a diamond saw under constant water irrigation, in a microtome (Isocut, Buheler Ltd, Lake Bluff, IL, USA). Sections of the specimens approximately 150 μm thick were obtained and

reduced by hand polishing to 100 μm ± 10. The sections were embedded in deionised water and mounted in glass-slides to be analysed in a polarized light microscope (DM LSP, Leica, Wetzlar GmbH, Germany), with a 20 x objective lens. The long axis of dentine tubules was orientated at 45º in relation to the crossed polarizers.

Images were taken to the computer, via digital camera, and analysed with the Image-Pro Plus software. The demineralised area around the restoration was determined in three zones of 40,000 μm^2 (100 μm width x 400 μm depth) apart from the restoration: Z1. 0-100 μm, Z2. 101-200 μm and Z3. 201-300 μm. The percentages of specimens with wall-lesion (WL) and inhibition zone (IZ) were also calculated. One blinded examiner performed all measurements.

Statistical analysis

The assumptions of equality of variances and normal distribution of errors were respectively checked with the Hartley and Shapiro-Wilks tests for the response variables tested. If necessary, data were submitted to transformation (12). Statistical approaches are described in Table 1. The significance level was set at 5 %. Analyses were performed with the SAS System 6.11 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

PHASE 1

Fluoride releasing data were transformed according to a root square function. The interaction between Material and Time was significant ($p = 0.0001$). Higher amounts of fluoride were released by control [+], A, B and C groups in the first day, decreasing throughout the days according to logarithmic equations ($p < 0.0001$ / $r^2 > 0.9$) (Graph 1). Groups control [-] and D did not release detectable amounts of fluoride during the period

tested (Graph 1). With regards to the cumulative amount of fluoride released, they were ordered as follows (from the higher to the lower level): control [+] > A > B > C > D = control [-] (Table 3).

PHASE 2

The interaction between Material and Distance was significant (p = 0.0001), for dentine demineralisation area. Within the factor Distance, the control [+] showed the lowest demineralised areas in Z1 and Z2, whereas no significant differences were found among groups A-D and control [-]. In Z3, no evident effect of the materials could be found, although A showed significant higher demineralisation area than other groups (comparisons in columns, Table 4). Within the factor Material, Z1 showed lower demineralisation area than in Z2 and Z3, which did not differ from each other, for all materials (comparisons in rows, Table 4). No WL was found. IZ was found in all groups, but in higher percentages in control [+] and A (Table 3).

DISCUSSION

All of the dental adhesives tested, excepting Tenure Quick and the control [-], released detectable amounts of fluoride during the pH cycling. However, the first null hypothesis (H1, table 1) was only accepted for Optibond Solo in the first 3 days of pH-cycling, when it was able to release fluoride in amounts similar to or higher than the glass-ionomer control. The fluoride-releasing rate of dental materials may be influenced by the solubility and type of the active component, as well as by the phase - organic or inorganic - in which it is added (6). Optibond Solo and One-up Bond F have fluorosilicate as the active component, which was able to provide a fluoride releasing behaviour quite similar to that of the glass-ionomer cement. Prime & Bond NT, which contains cetylamine hydrofluoride,

was also effective in release fluoride. Comparisons based on the compositions of the tested dental adhesives were limited by the huge differences in their overall formulations and also by the lack of data for some adhesives. The implications of the fluoride released on the physical properties of the dental adhesives are not known and should be further tested. Depending on the mechanism involved, not only the fluoride-releasing rate but also the physical properties of the material may be affected (6).

The results of the 1st phase suggested some cariostatic potential for the fluoride releasing adhesives. Nevertheless, this potential was not effective on the 2nd phase of this study and the 2nd null hypothesis formulated (H2, Table 1) was rejected for all dental adhesives in zones 1 and 2. In fact, although the dentine/restoration interface area was the same among groups, the volume of the material was not, since cavities of the control [+] group were entirely restored with glass-ionomer cement. This evidently implied that more fluoride was available to the latter group, making the comparison experimentally unfavourable to the dental adhesives. However, that was done to resemble the clinical situation and to provide a comparative parameter.

The artificial caries regimen used might also have contributed to reduce the cariostatic effect of the dental adhesives, by diluting the fluoride amounts released by them in the demineralising and remineralising solutions. In clinical conditions, it can be assumed that this diluting effect would be minimized by the presence of the dental plaque. Additionally, this caries regimen may be considered aggressive, since extensive demineralisation was observed in the control [-] group. Possibly, in less severe conditions, some effect could be evidenced. However, results comparable to that obtained to the glass-ionomer should not be expected, since in a previous study (13) with a less aggressive artificial caries model no evident cariostatic effect was found at 50 μm from the restoration when using the Prime & Bond NT dental adhesive, even associated to a

fluoride-containing composite. In contrast, an inhibition area was found up to 150 μm for the glass-ionomer cement (13). Although the fluoride released from dental adhesives seemed to be not enough to prevent secondary caries in root dentine, it could not be concluded that it is worthless. Fluoride releasing dental adhesives might have some effect in the improvement of the acid-resistance of the dentine cavity margins (8,9,14).

Even though it is not well defined whether the development of a cavity wall lesion may or may not be a clinical problem (15), no wall lesion (WL) was detected in any group. The constant development of the dental adhesives has allowed the achievement of reliable sealing capacity to dentine, even when submitted to artificial cariogenic challenges and also to thermal stresses, as done in this study. This result corroborated to that reported by ITOTA *et al.* (14), with regards to the importance of the use of reliable dental adhesives to avoid the development of WL. In addition to that, demineralisation inhibition zones (IZ) were found in all groups (Fig 1), being the percentage of IZ of Optibond Solo even similar to that of the control glass-ionomer group. These data did not imply necessarily in cariostatic effect, as showed by the higher demineralisation areas found to the dental adhesives in Z1 and Z2, although might suggest the importance in using a fluoride-containing dental adhesive to maintain the integrity of the cavity margins.

It was not clear if the IZ was only related to the fluoride since Tenure Quick and control [-], which did not release detectable amounts of fluoride, also presented this zone - but in lower percentages. Perhaps, the use of more sensitivity techniques to detect fluoride (16) could explain this achievement, since Tenure Quick was expected to release F (according to the manufacturer), and Single Bond was previously reported to release small amounts of F (17), even though it has not been stated by the manufacturer. Although a trend between higher amounts of fluoride released and higher percentages of IZ could be suggested (Table 3), other factors should also be considered. The IZs found in this study

could also be related to the formation of an effective resin-dentine interdifusion zone - or hybrid layer - that is supposed to be more acid-resistant than dentine (18). In fact, the tested fluoride releasing dental adhesives were able to keep the integrity of the cavity wall, although they could not prevent secondary caries.

It could be concluded that although some dental adhesives could release fluoride they were not able to inhibit the development of secondary caries on root dentine surfaces in the conditions of this study.

ACKNOWLEDGEMENTS

Based on a thesis submitted by the first author to the Faculty of Dentistry of Piracicaba, University of Campinas (UNICAMP), in partial fulfilment of the requirements for the Doctoral degree in Clinical Dentistry (Restorative Dentistry) degree. The authors would like to express their gratitude to Dr. Lourenço Correr Sobrinho, Department of Dental Materials, UNICAMP, for the facilities; and to Mrs Mariza de Jesus C. Soares and Mr José Alfredo da Silva, Laboratory of Biochemistry, UNICAMP, for the technical assistance. This investigation was supported by Research Grant # 00/14278-0 from the State of São Paulo Research Foundation / FAPESP, São Paulo, Brazil.

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TABLES

Table 1. Experimental designs and statistical approaches.

	Phase 1	Phase 2
Factors	Material: 6 levels (*plot*) and Time: 8 levels (*subplot*)	Material: 6 levels (*plot*) and Distance: 3 levels (*subplot*)
Design	Factorial 6 x 8 / randomised complete block and *split-plot* design	Factorial 6 x 3 / randomised complete block and *split-plot* design
Block	Period to make the specimens	Period to restore the slabs
Restrictions in the randomisation	Provided by the factor Time	Provided by the factor Distance
Null hyphotesis	H1: adhesives release fluoride as a glass-ionomer	H2: adhesives inhibit secondary caries as a glass-ionomer
N	5	16
Response variables	Fluoride release: μg F/cm^2 (daily and cumulative)	Demineralised area: μm^2; % of WL* and IZ*
Statistical analysis	Anova *split-plot*/Tukey test (F daily release); Anova/Tukey test (F cumulative release)	Anova *split-plot*/Tukey test (demineralised area); *t*-test for %s (WL and IZ)

*WL: wall lesion; IZ: inhibition zone.

Table 2. Types, batch numbers and manufacturers of the dental materials used.

Brand Name	Groups	Batch #	Composition*	Manufacturer
Ketac-fil *Plus*	GIC control [+]	Powder: 013 Liquid: 0023	Powder: Strontium fluorosilicate glass, Al, La; Liquid: Copolimer acid.	Espe GmbH Seefeld, Germany
		Nail varnish: --	--	Colorama - CEIL, São Paulo, SP, Brazil
Optibond Solo Plus	F-DA	Bottle: 107293 H_3PO_4 37.5%: 05383	Ethyl alcohol, Alkyl dimethacrylate resins, Barium aluminoborosilicate glass, fumed silica, sodium hexafluorosilicate	Kerr Corp. Orange, CA, USA
One Up bond F	F-DA	Box: 00231E A: 034 / B: 531	A: Methacrylates; B: Methacrylates, Water, Fluoroaluminosilicate, Glass filler	Tokuyama Corp. Tokyo, Japan
Prime & Bond NT	F-DA	Bottle: 0105001127 H_3PO_4 34%: 68274	Di and Trimethacrylate resins, PENTA, Nanofillers - amorphous silicon dioxide, Photoinitiators, Stabilizers, Cetylamine hydrofluoride, Acetone	Dentsply Caulk Milford, DE, USA
Tenure Quick	F-DA	Bottle: C320010022 H_3PO_4 37%: C331010006	Dimethacrylate resin, Methacrylate resin, Acetone, PMDM	Den-Mat Corp. St. Maria, CA, USA
Single Bond	N-DA control [-]	Bottle: 9 DE H_3PO_4 35%: 1WR	Water, Ethanol, HEMA, BIS-GMA, Dimethacrylates	3M Dental Products St. Paul, MN, USA
Filtek Z250	CR	Seringe: 1LU Color: A2	Zirconia, silica, TEGDMA, UDMA BIS-EMA	3M Dental Products St. Paul, MN, USA

GIC: glass-ionomer cement; N-DA: non-fluoridated dental adhesive; F-DA: fluoridated dental adhesive; CR: composite resin.

* Based on information provided by the manufacturers (HEMA = hydroxiethyl methacrylate; BIS-GMA = bisphenol glycidyl methacrylate; TEGDMA = tetraethyleneglycol dimethacrylate; UDMA = urethane dimethacrylate; BIS-EMA = Bisphenol A polyetheylene glycol diether dimethacrylate; PENTA = dipentaerythritol penta acrylate monophosphate; PMDM = pyromellitic dimethacrylate).

Table 3. Cumulative fluoride released means (standard-deviation) and percentages of specimens with wall-lesions and inhibition-zones.

Groups	Cumulative F^- release $\mu g\ F/cm^2$	WL %	IZ %
Ketac-fil *Plus*	29.19 (2.07) a	0	93.7 a
Optbond Solo Plus	25.81 (0.84) b	0	68.7 ab
One Up bond F	5.42 (0.43) c	0	46.6 bc
Prime & Bond NT	3.56 (0.34) d	0	56.2 bc
Tenure Quick	-1.30 (0.07) e	0	31.2 bc
Single Bond	-1.05 (0.05) e	0	37.5 c

*WL: wall lesion; IZ: inhibition zone.

Table 4. Mean (standard deviation) of the demineralisation areas (μm^2), in the three zones (100 μm width and 400 μm high) apart from the restoration.

Groups	Z1		Z2		Z3	
[+]. Ketac-fil *Plus*	a 8492.91 (5251.04)	A	b 19921.57 (5250.90)	A	b 21077.34 (4490.68)	A
A. Optbond Solo Plus	a 16251.82 (4438.92)	B	b 23881.95 (4014.07)	B	b 24458.59 (3114.94)	B
B. One Up bond F	a 19288.54 (3437.25)	B	b 24010.32 (2539.20)	B	b 23836.63 (2525.05)	AB
C. Prime & Bond NT	a 16489.39 (4601.29)	B	b 24505.5 (3533.15)	B	b 23978.81 (3417.16)	AB
D. Tenure Quick	a 20336.37 (4902.93)	B	b 24270.28 (2878.03)	B	b 23665.49 (3133.59)	AB
[-]. Single Bond	a 18417.86 (2750.80)	B	b 23103.24 (2100.32)	B	b 22967.04 (1931.59)	AB

Statistical differences are expressed by different small letters in rows and capital letters in columns ($\alpha = 0.05$).

FIGURES

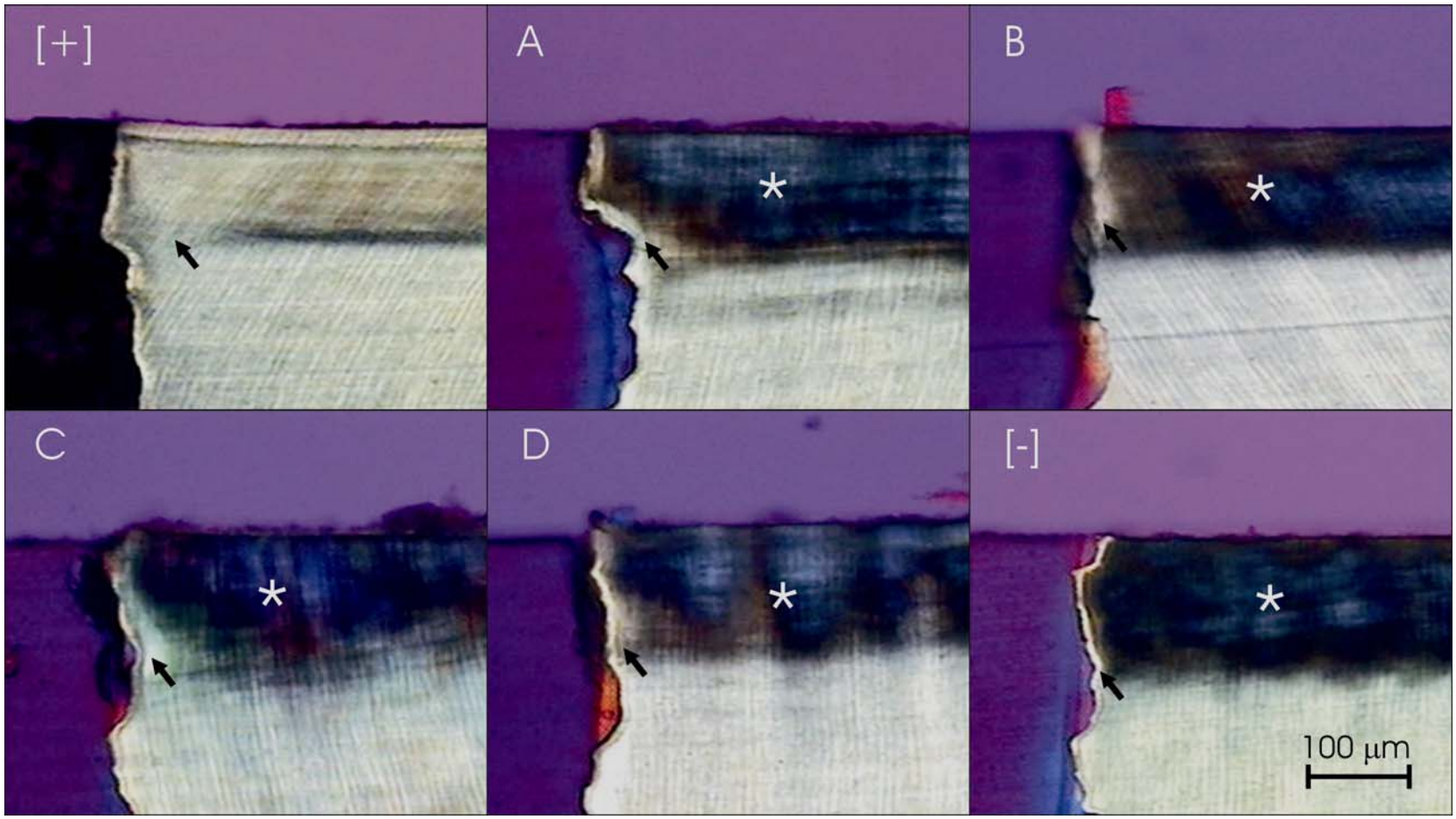

Figure 1. Polarized transmitted light micrographs of: [+]. glass-ionomer, A. Optibond Solo, B. One-up Bond F, C. Prime & Bond NT, D. Tenure Quick and [-]. Single Bond. Note extensive demineralisation areas in dentin around restoration (asterisks) in all groups, except in [+]. Inhibition Zone (arrows) can be seen in the cavity margin of all groups.

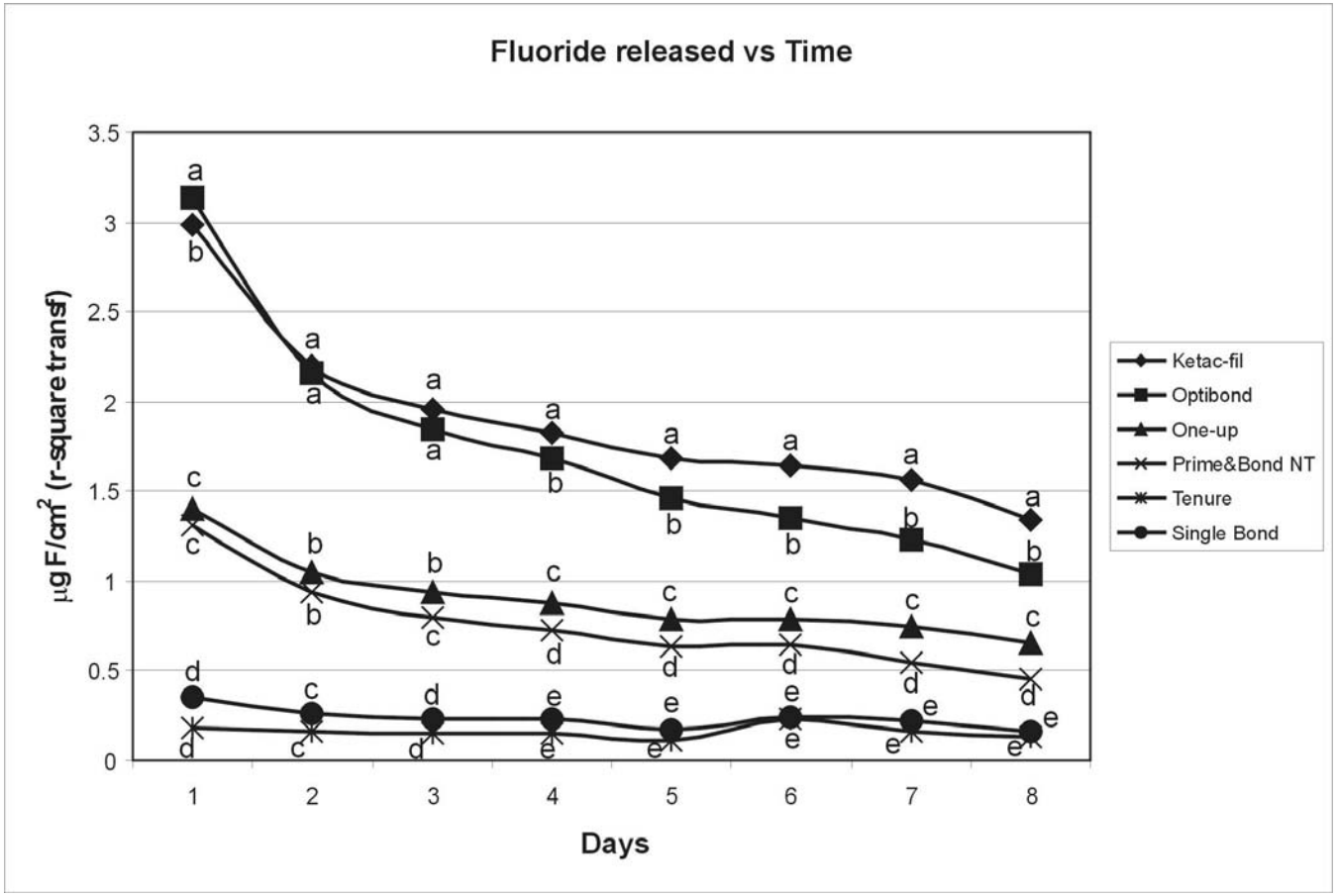

Figure 2. Fluoride behaviors. Different letters imply in statistical differences among groups, within each day. Logarithmic equations were fit to: Ketac-fil [+], Optibond, One-up and Prime&Bond NT ($p<0.0001$; $r^2 > 0.9$), showing a higher fluoride release in the initial days, which decreased throughout the time. Tenure and Single Bond [-], did not release detectable amounts of fluoride.

CAPTIONS TO TABLES AND FIGURES

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Table 2. Types, batch numbers and manufacturers of the dental materials used.

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3.2. CAPÍTULO 2

OPERATIVE DENTISTRY, 27(5): 225-228, 2002 (ANEXO 3).

EXTENT OF THE CARIOSTATIC EFFECT ON ROOT DENTIN PROVIDED BY FLUORIDE-CONTAINING RESTORATIVE MATERIALS

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TITLE

Extent of the cariostatic effect on root dentin provided by fluoride-containing restorative materials

RUNNING TITLE

Extent of the cariostatic effect on root dentin provided by restorations

CLINICAL RELEVANCE

The extent to which fluoride is effective around a glass-ionomer cement and a resin-modified glass-ionomer are estimated to be about 0.3 and 0.15 mm, respectively, in root dentin. This could be important for reducing secondary root caries development.

SUMMARY

The aim of this study was to evaluate the extent of the cariostatic effect on root dentin provided by four fluoride-containing restorative systems: Ketac-fil/Espe [Ke], Fuji II LC *Improved*/GC Corp. [Fj], Dyract AP/Dentsply [Dy] and Surefil/Dentsply [Su], and one without fluoride: Z250/3M [control]. Ninety-five bovine root dentin fragments (5.0x6.0-mm) were obtained, embedded in polyester resin and planed. Cavities (1.5x3.5x1.0-mm) were made and restored by the five restorative systems (n=19), according to the manufacturers' instructions, in a randomized complete block design. After 24-hours, the dentin/restoration surface was polished. The restoration surface and an adjacent area of 3.0x3.0-mm were demarcated and submitted to a pH-cycling model. Dentin surface Knoop microhardness values were obtained (5.0-g, 5.0-s) for ten distances: 50, 100, 150, 300, 600, 900, 1200, 1500, 1800, 2100-μm from the margin of the restoration. The dentin microhardness means for each of the restorative materials at each distance were considered by the ANOVA multi-factor split-plot method. The interaction between restorative system and distance was statistically significant (pvalue<0.05). The Tukey test and the regression analysis showed that the means of [Ke] and [Fj] were similar up to the distance 300-μm, the [Ke] means being higher than the [control] at distances 50, 100, 150 and 300-μm. The [Fj] means were higher than the [control], at distances 50, 100 and 150-μm. The microhardness means of [Dy] and [Su] were not statistically different from the [control], remaining steady throughout the studied distances. The study concluded that the extent of the cariostatic effect on root dentin was 300-μm for [Ke] and 150-μm for [Fj]. [Dy] and [Su] did not show any cariostatic effect.

INTRODUCTION

In vitro studies have shown that fluoride-containing restorative materials can inhibit the development of root surface caries adjacent to restorations (Tam, Chan & Yim, 1997; Pereira & others, 1998; Dionysopoulos & others, 1998; Torii & others, 2001). This effect is attributed to fluoride ions released from these materials, which may act mainly by inhibiting dentin demineralization and/or by enhancing its remineralization process (Featherstone, 1994). In this way, the amount of fluoride released from the restoration probably explains the differences in *in vitro* secondary caries inhibition of various fluoride-containing restorative materials. Conventional glass-ionomer cements (GIC) generally release an equivalent or higher amount of fluoride than resin-modified glass-ionomers (RMGI), and both release more fluoride than polyacid-modified composite resins (PMCR) and fluoride containing-composite resins (FCR) (Suljak & Hatibovic-Kofman, 1996; Vermeersch, Leloup & Vreven, 2001).

However, the extent of the cariostatic effect on root dentin surface has not been evaluated. Although the use of fluoride-containing restorative materials is frequently indicated for preventing caries development at dental restoration margins, it is common to observe other sites of high risk of root caries through easy dental plaque accumulation (Erickson & Glasspoole, 1995), like proximal root surfaces (Schüpbach, Lutz & Guggenheim, 1992) and root surfaces close to gingival margins (Lynch & Beighton, 1994). Therefore, can fluoride-containing restorations extend their cariostatic effect as far as this? There is evidence that glass-ionomer cement not only prevents caries formation at the cavity wall but also inhibits lesion progression in enamel at a considerable distance from the restoration (Tantbirojn, Douglas & Versluis, 1997). Nevertheless, it is not known whether glass-ionomer cement restorations placed in root dentin can also show their

protective effect and, furthermore, whether other fluoride releasing restorative materials, like RMGI, PMCR and FCR, are capable of behaving in the same way.

This *in vitro* study was designed to evaluate the extent of the cariostatic effect of five restorative systems: a glass-ionomer cement, a resin-modified glass-ionomer, a polyacid modified composite resin, a composite resin with fluoride and a composite resin without fluoride (control), on root dentin surfaces adjacent to restorations. Specifically, this study tested whether there were any differences in dentin microhardness adjacent to restorative systems, up to 2.1-mm from the restoration margin.

METHODS AND MATERIALS

Experimental design

The factors under study were Restorative system at five levels (Table 1) and Distance from restoration at ten levels: 50, 100, 150, 300, 600, 900, 1200, 1500, 1800, 2100-μm. The experimental units were 95 root dentin fragments (n=19), restored in 19 blocks of 5 fragments each - one fragment for each restorative system. Within each block, the order in which the five materials were used to restore the fragments was randomly determined. A randomized complete block design was used to systematically control the variability arising from known nuisance sources (Montgomery, 1991). As it was not possible to completely randomize the order of the Distance level analysis, a randomization restriction was considered, characterizing a factorial 5x10 *split-plot* design (Montgomery, 1991). The three basic principles of experimental design: replication, randomization and blocking were employed (Montgomery, 1991). The continuous quantitative response variable was the Knoop microhardness value.

Tooth fragment preparation

Ninety-five bovine incisor teeth were collected and stored in a 10% neutral buffered formalin solution, until they were used. They were cleaned by means of a hand-scaler and a non-fluoride polishing paste. Defective root surfaces were discarded. The crown and the apical region of the root were cut-off with a double-faced diamond disc (# 7020 - KG Sorensen, São Paulo, SP, Brazil) in a low-speed handpiece, to obtain 95 root fragments with approximately 5.0-mm width, 6.0-mm length and 2.0-mm thickness (Fig 1.1). These fragments were embedded in polyester resin (5061 N - Cray Valley Ltda, Taboão da Serra, SP, Brazil) and sanded with a water-cooled mechanical grinder (Maxigrind - Solotest, São Paulo, SP, Brazil), using a # 400, 600 and 1000-grit Al_2O_3 abrasive paper (Carborundum Abrasivos, Recife, PE, Brazil), in order to expose at least 4-mm width and 6-mm length area of dentin surface.

A cavity having 1.5-mm width, 3.5-mm length and 1.0-mm depth was made using a diamond bur (# 2096 - KG Sorensen, São Paulo, SP, Brazil) in a high-speed handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil), under a constant water-spray coolant (Fig 1.2).

Restoration and polishing procedures

The prepared tooth fragments were restored according to the randomized complete block design. Nineteen blocks, each with 5 fragments, were made. The restorative techniques recommended by manufacturers were followed (Table 2) (Fig 1.3). The restored tooth fragments were individually immersed in artificial saliva at 37 ± 1ºC for 24-hours. After that, the restored surface was polished with a water-cooled mechanical grinder (Maxigrind - Solotest, São Paulo, SP, Brazil) with # 1000 Al_2O_3 abrasive paper

(Carborundum Abrasivos, Recife, PE, Brazil). The restored tooth fragments were re-immersed in artificial saliva at 37 ± 1ºC, for 24-hours.

pH-cycling model

The dentin surfaces were covered with an acid resistant varnish (Colorama - CEIL, São Paulo, SP, Brazil) leaving exposed only an area of 1.5-mm width x 3.0-mm length of the restorations and an adjacent area of 3.0-mm x 3.0-mm of dentin surface (Fig 1.4).

The specimens were individually submitted to demineralizing (De) (2.0-mM Ca, 2.0-mM P in a buffer solution of 74-mM of acetate at pH 4.3) and remineralizing (Re) (1.5-mM Ca and 0.9-mM P in a buffer solution of 20.0mM of Tris (hydroxymethil)-aminomethane at pH 7.0) solutions, similar to that proposed by Featherstone & others (1986) and modified by Serra & Cury (1992) for enamel substrate. As dentin has a lower mineral content than enamel, it was necessary to previously determine the number of cycles and the specimen immersion-time in each solution, in order to make it possible to measure the Knoop surface microhardness after the artificial cariogenic challenge. This explains the choice of two 24-hour cycles: 30-min in demineralizing solution, 3-hours in a remineralizing solution, 30-min in De and 20-hours in Re (Fig 1.5).

Microhardness assessment

The surface microhardness values (*KHN*) were obtained in a microhardness tester (HMV 2000 - Shimadzu, Japan) with a Knoop diamond and a 5-g static-load, which was applied for 5-seconds. Ten indentations were sequentially made at 50, 100, 150, 300, 600, 900, 1200, 1500, 1800, 2100-μm from the margin of the restoration (Fig 1.6).

Statistical analysis

A multi-factor analysis of variance (ANOVA) (α = 0.05) for *split-plot* design was applied. A study of the interaction among the factors analyzed (Restorative system, Distance and Block) was made. The interaction of particular interest was Restorative system x Distance. Multiple Comparisons Tukey test (α= 0.05) was chosen to check differences in means within the factor Distance, and a regression analysis was chosen to show the behavior along the distances within the factor Restorative system. The analysis was performed with the SAS System 6.11 (SAS Institute Inc., Cary, NC, 27513-2414) and the Curve Expert 1.3 (www.ebicom.net/~dhyams/cvxpt.htm) software.

RESULTS

The data did not present homogeneity of variances. In order to stabilize them, they were submitted to a reciprocal transformation (y = 1/x).

The means (standard deviation) of the transformed Knoop microhardness values of each of the fifty groups (10 distances x 5 restorative systems) are given in table 3. The ANOVA for *split plot* showed a significant interaction between Restorative system and Distance (p_{value} = 0.001). Within the factor Distance, the Tukey test showed that Ketac-fil and Fuji II LC had similar microhardness values up to the distance 300-μm. Ketac-fil had higher microhardness values when compared to the control group (Z250), up to the distance 300-μm; and Fuji II LC differed from the control up to the distance 150-μm. Beyond these distances, both did not differ from the control. Dyract and Surefil did not differ from the control at any of the distances. For Dyract at the distances 600, 1200 and 2100-μm the microhardness was significantly lower than the control. Only at distance 1800-μm, all restorative systems showed no significant difference from each other.

Within the factor Distance, regression analysis allowed the characterization of behavior for each restorative system along the distances from the restoration margins. Ketac-fil and Fuji II LC had similar behavior, which can be represented by logarithmic curves (y = 0.0192 + 0.0091*lnx, r = 0.97; and y = 0.0297 + 0.0076*lnx, r = 0.97, respectively), with high microhardness values close to the restoration (Graphs 1 and 2). Surefil behavior could also be represented by a logarithmic curve (y = 0.0713 + 0.0028lnx, r = 0.89), however with visually lower microhardness values than Ketac-fil and Fuji II LC, at close distances (Graph 3). For Dyract the best curve to explain its behavior was the linear (y = 0.0919 + 3.28e-006x, r = 0.85) (Graph 4). For Z250 a linear curve was also adjusted (y = 0.0870 + 5.61e-008x), but there was no casual relationship between microhardness and distance (r = 0.02) (Graph 5).

DISCUSSION

Fluoride-releasing restorative materials, like GIC, RMGI and PMCR have shown caries inhibition capacity in laboratory studies (Tam & others, 1997; Pereira & others, 1998; Dionysopoulos & others, 1998; Millar, Abiden & Nicholson, 1998). However, they have not confirmed this behavior in clinical trials (Levy & others, 1989; Kaurich & others, 1991). Thus, it has not yet been proven that fluoride-releasing restorative materials are capable of preventing secondary caries (Erickson & Glasspoole, 1995; Randall & Wilson, 1999). Several aspects should be considered in order to try to explain this fact and, within them, the extent of the cariostatic effect can be evidenced. Clinically, the cariostatic effect just close to the edge of the restoration may not be sufficient to prevent secondary caries development. Apart from the presence of the restoration, other factors can also contribute to increasing the risk of root caries adjacent to restorations, such as

the nearness to gingival margins and/or to proximal surfaces (Erickson & Glasspoole, 1995; Mjör & Toffenetti, 2000). Since secondary caries occurs by the development of cariogenic conditions adjacent to restorations (Thylstrup, 1998), it is important to study not only the cariostatic effect just at the dentin margin of the cavity, but also on dentin surfaces along the margins of restorations.

Differences among restorative systems were evaluated at ten distances along the restoration margin. The dentin microhardness values adjacent to Ketac-fil were higher than the control up to distance of 300-μm. For Fuji II LC the higher microhardness values were observed only at distances 50, 100 and 150-μm. Probably, the rate of fluoride ions released may explain this result, since previous studies have shown that Ketac-fil releases more fluoride than Fuji II LC (Vermeersch & others, 2001).

Surefil behaved in the same way as the control along all distances. The ability of Surefil in inhibiting artificial caries development was not observed. This could be explained by the relatively low fluoride ion release from fluoride-containing composite resins to the surrounding oral micro-environment (Erickson & Glasspoole, 1995; Arends, Dijkman & Dijkman, 1995; Karantakis & others, 2000). The dentin microhardness values adjacent to Dyract were similar to the control, but at distances 600, 1200 and 2100-μm, they have an unexpected behavior, being statistically lower than the control Z250. Some cariostatic effect was expected for Dyract, since in demineralizing and remineralizing solution the water-free acid group components are expected to ionize and to interact with the basic glass components, developing an acid-base reaction with fluoride release (Eliades, Kakaboura & Palaghias, 1998; Meyer, Cattani-Lorente & Dupuis, 1998). However, Dyract performed equally or worse than the control group. This result did not

confirm that reported in the literature, where Dyract demonstrated some caries inhibition effect *in vitro* (Dionysopoulos & others, 1998; Millar & others, 1998).

Beyond distance 300-μm it was presumed that all groups of restorative materials would behave in the same way, but this occurred only at distance 1800-μm. The unexpected behavior of Dyract contributed to this, since Ketac-fil, Fuji II LC and Surefil did not differ from the control after the distance 300-μm.

Regression analysis was helpful in explaining the behavior of dentin microhardness response as a function of distances, according to mathematical models. Logarithmic curves (Graphs 1, 2 and 3) allow the supposition that higher amounts of fluoride released from the restorations may interfere with caries development in dentin close to the restorative material. Thus, higher microhardness values could be observed at initial distances in the Ketac-fil and Fuji II LC graphs. For Surefil the microhardness values were not highly pronounced close to restoration. Probably this material had a low cariostatic effect, which was not evidenced at the distances analyzed in this study. The absence of statistically significant differences between Surefil and control at all distances confirm this finding. Throughout distances, the microhardness values dropped until they established a constant level, where, presumably, the fluoride cannot extend its effect.

Linear curves were adjusted for Dyract (Graph 4) and Z250 (Graph 5). For the latter a low correlation coefficient was obtained ($r = 0.02$) showing no correlation between microhardness and distance. However, the aspect of plotted data allowed the supposition that the microhardness of dentin adjacent to Z250 remained steady throughout the distances in this study. For these materials it was concluded that fluoride could not influence dentin microhardness by inhibiting demineralization and/or enhancing remineralization processes.

In fact, Ketac-fil and Fuji II LC showed an extent of cariostatic effect, that is they were capable of inhibiting artificial caries development beyond the restoration margins. However, this *in vitro* study was not able to entirely reproduce clinical conditions of cariogenic challenge. The effects of dental plaque - as a mechanical barrier (Erickson & Glasspoole, 1995) - and of saliva - as a fluoride disperser by continual replenishment (Erickson & Glasspoole, 1995; Tantbirojn & others, 1997) - were not considered in the current study, and they seem to be related to the extent of cariostatic effect. Further studies reproducing clinical conditions are necessary to confirm the findings obtained in the current work. *In situ* models appear to be a good option. However, the results obtained in this study are useful for distinguishing the cariostatic effect among different fluoride-containing restorative systems.

Another interesting aspect to be considered is that this analysis was performed four days after the restorations were made. Although various fluoride-containing restorative materials have different rates and durations of fluoride release, most of the major fluoride release usually takes place within the first seven days (Hsu & others, 1998; Vermeersch & others, 2001). Thus, the fluoride effect might be amplified by the experimental conditions. A study will be carried out evaluating the extent of the cariostatic effect of aging restorations.

To answer the question that encouraged the initiation of this study, Dyract and Surefil, did not show a cariostatic effect. The extent to which fluoride was effective around Ketac-fil and Fuji II LC was estimated to be about 300 and 150-μm, respectively. Although this extent could not be so large as to prevent root caries far from a restoration, it is important for reducing secondary root caries development close to the restoration margin.

CONCLUSION

The extent of the cariostatic effect on root dentin was 300-μm for Ketac-fil and 150-μm for Fuji II LC *Improved*. Dyract AP and Surefil did not show any cariostatic effect.

ACKNOWLEDGEMENTS

Based on a thesis submitted in partial fulfillment of the requirements for the ScD degree, State University of Campinas (UNICAMP). The authors would like to express their gratitude to Dr. Jaime A. Cury, Department of Physiologic Sciences, UNICAMP; to Dr. Altair A. Del Bel Cury and Dr. Renata C.M. Rodrigues, Department of Prosthesis and Periodontology, UNICAMP; to Dr. Lourenço C. Sobrinho, Department of Dental Materials, UNICAMP and to Dr. Antonio Luiz Rodrigues Jr, Discipline of Bio-statistics and Scientific Methodology, State University of São Paulo (UNESP), for the facilities.

Supported by FAPESP (Process # 00/14278-0).

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TABLES

Table 1. Restorative systems tested.

Brand Name	Type	Batch #	Manufacturer
Ketac-fil *Plus*	GIC	Powder: FW0055787 Liquid: FW0056696 Ketac Conditioner: 0004	Espe GmbH Seefeld, Germany
		Heliobond*: 0120598	Vigodent S.A. Rio de Janeiro, RJ Brazil
Fuji II LC *Improved*	RMGI	Powder: 160291 Liquid: 260191 GC Dentin Conditioner: 201241	GC Corporation. Tokyo, Japan
		Heliobond*: 0120598	Vigodent S.A. Rio de Janeiro, RJ Brazil
Dyract AP	PMCR/AS	9904001505 Prime&Bond NT: 9811001097 H_3PO_4 34%: 990417	Dentsply Caulk Milford, DE, USA
Surefil/Prime & Bond NT	FCR/FAS	990119 Prime&Bond NT: 9811001097 H_3PO_4 34%: 990417	Dentsply Caulk Milford, DE, USA
Filtek Z250/Single Bond	CR/AS	9BX Single Bond: 9CY H_3PO_4 35%: 9PD	3M Dental Products St. Paul, MN, USA

* Used as surface protector.
GIC = conventional glass-ionomer cement; RMGI = resin-modified glass-ionomer; PMCR = polyacid-modified composite resin; AS = adhesive system; FCR = fluoride-containing composite resin; FAS = fluoride-containing adhesive system; CR = composite resin.

Table 2. Restorative techniques used according to manufacturers' recommendations.

Restorative System	Dentin Pretreatment	Dispensing and Mixing	Insertion	Light Curing [6]	Surface Protection
Ketac-fil *Plus* [1]	Ketac Conditioner [1]	Powder/Liquid ratio of 3/2 (g/g) Manually	Centrix Syringe [5]	None	Heliobond [7]
Fuji II LC *Improved* [2]	GC Conditioner [2]	Powder/Liquid ratio of 3/2 (g/g) Manually	Centrix Syringe [5]	20 seconds	Heliobond [7]
Dyract AP/Prime Bond NT [4]	H_3PO_4 34% [4] Prime & Bond NT Adhesive [4]	None	None	40 seconds	None
Surefil/Prime & Bond NT [4]	H_3PO_4 34% [4] Prime & Bond NT Adhesive [4]	None	None	40 seconds	None
Filtek Z250/Single Bond [3]	H_3PO_4 35% [3] Single Bond Adhesive [3]	None	None	30 seconds	None

1 = Espe; 2 = GC Corp; 3 = 3M Dental Products; 4 = Dentsply Caulk; 5 = Centrix Inc; 6 = Light intensity ranging from 550~600 mW/cm^2; 7 = Vigodent.

Table 3. Means and standard deviation of transformed (y = 1/x) microhardness values.

	50	100	150	300	600	900	1200	1500	1800	2100
Ketac	0.05092 ^{A}	0.06153 ^{A}	0.06747 ^{A}	0.07647 ^{A}	0.07983 ^{A}	0.08255 ^{A}	0.08495 ^{A}	0.08283 ^{A}	0.08586 ^{A}	0.09019 ^{AB}
	0.01258	0.01388	0.01617	0.01864	0.01408	0.01500	0.01404	0.01477	0.01399	0.01387
Fuji II LC	0.05692 ^{A}	0.06385 ^{A}	0.06911 ^{A}	0.07774 ^{AB}	0.08028 ^{A}	0.08323 ^{AB}	0.08463 ^{A}	0.08440 ^{A}	0.08500 ^{A}	0.08670 ^{A}
	0.01443	0.01451	0.01700	0.01242	0.01299	0.01418	0.01433	0.01437	0.01477	0.01507
Surefil	0.08085 ^{B}	0.08345 ^{B}	0.08584 ^{B}	0.08968 ^{B}	0.08964 ^{A}	0.09416 ^{BC}	0.09156 ^{AB}	0.09181 ^{AB}	0.09000 ^{A}	0.09136 ^{AB}
	0.01808	0.01511	0.01616	0.01588	0.01731	0.01169	0.01739	0.01581	0.01854	0.01760
Z250	0.08591 ^{BC}	0.08408 ^{B}	0.08940 ^{B}	0.08816 ^{B}	0.08777 ^{A}	0.08772 ^{ABC}	0.08656 ^{A}	0.08890 ^{AB}	0.08580 ^{A}	0.08682 ^{A}
	0.01398	0.01500	0.01298	0.01503	0.01672	0.01781	0.01689	0.02124	0.02116	0.01943
Dyract	0.09242 ^{C}	0.09147 ^{B}	0.09145 ^{B}	0.09126 ^{B}	0.09574 ^{B}	0.09720 ^{C}	0.09780 ^{B}	0.09546 ^{B}	0.09675 ^{A}	0.09859 ^{B}
	0.01113	0.01119	0.01062	0.01435	0.01249	0.01057	0.01158	0.01308	0.01359	0.01251

Statistical differences are expressed by different letters following the means, in COLUMNS ($p < 0.05$).
l.s.d. = 0.0109653.

ILLUSTRATIONS

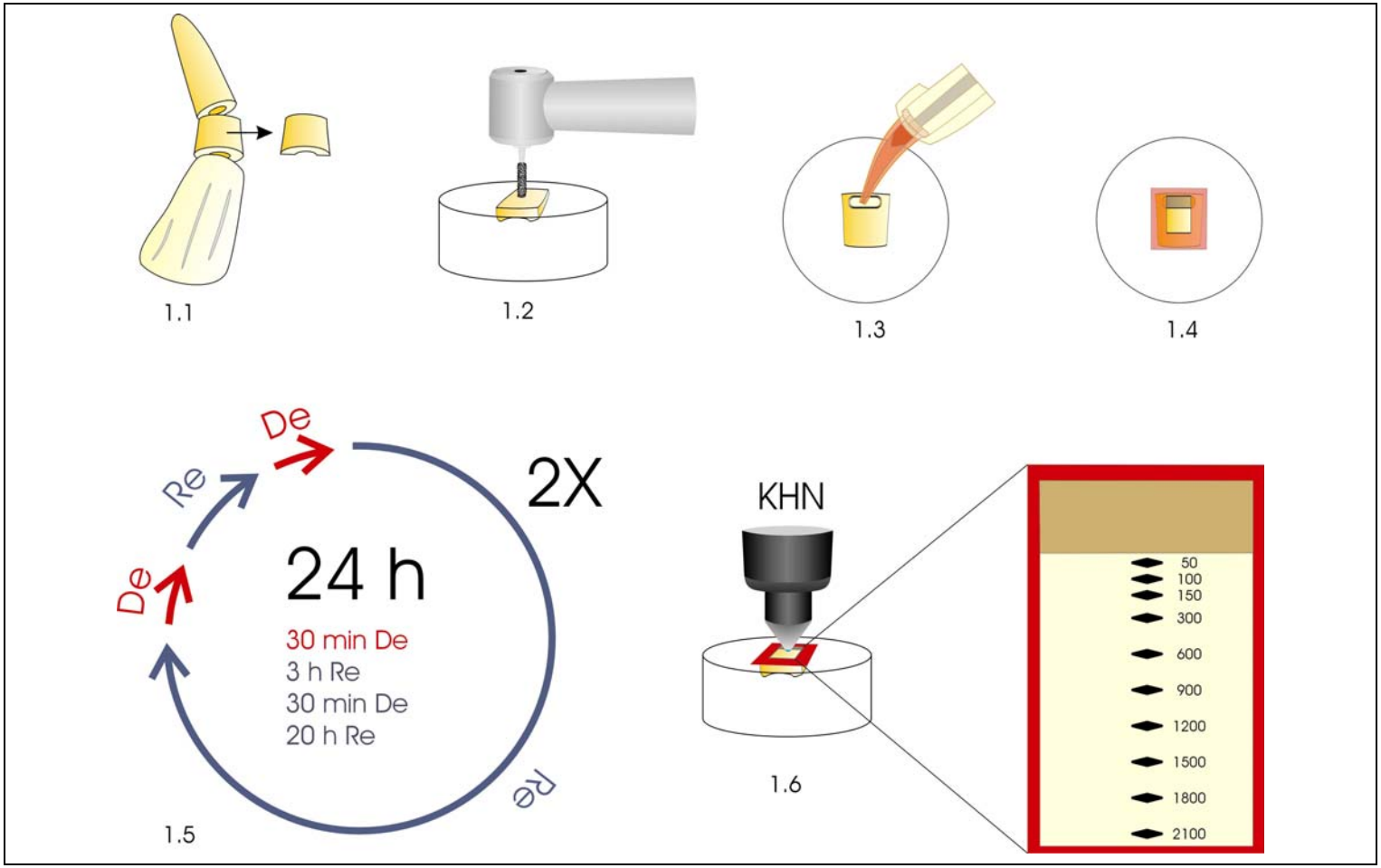
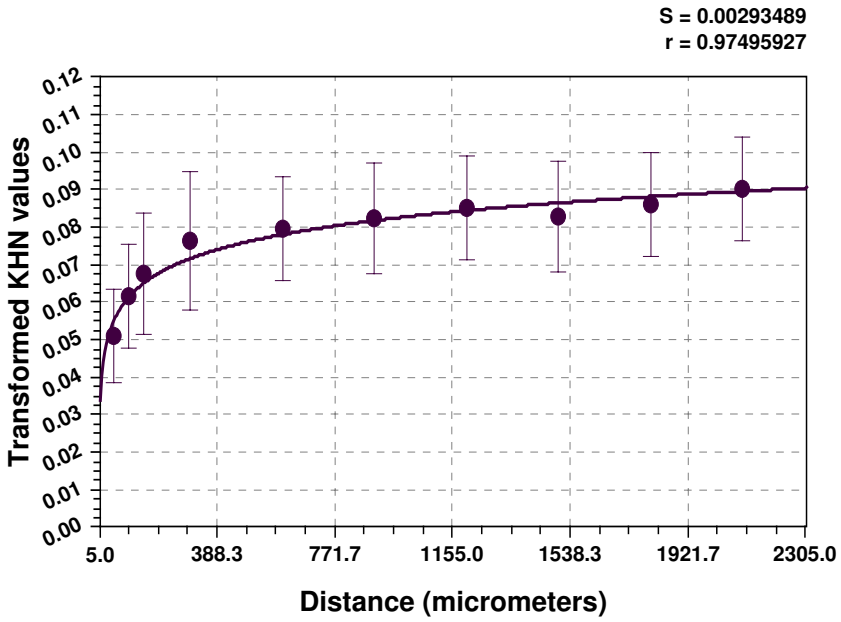


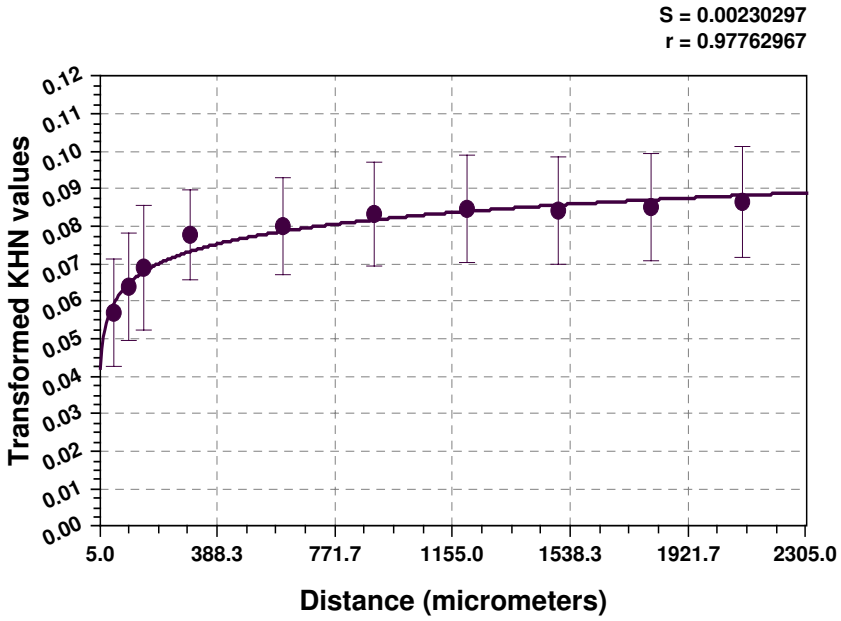
Fig 1. 1. Section of a bovine root dentin fragment; 2. Cavity preparation (1.5-mm width, 3.5-mm length and 1.0-mm depth) in an embedded and sanded root fragment; 3. Restoration procedures according to manufacturers' instructions; 4. Restoration (1.5-mm width x 3.0-mm length) and dentin (3.0-mm x 3.0-mm) surface areas left exposed to artificial caries development; 5. Artificial caries challenge based on pH cycling model; 6. The indentations made at ten distances to obtain the Knoop microhardness values.

GRAPHS

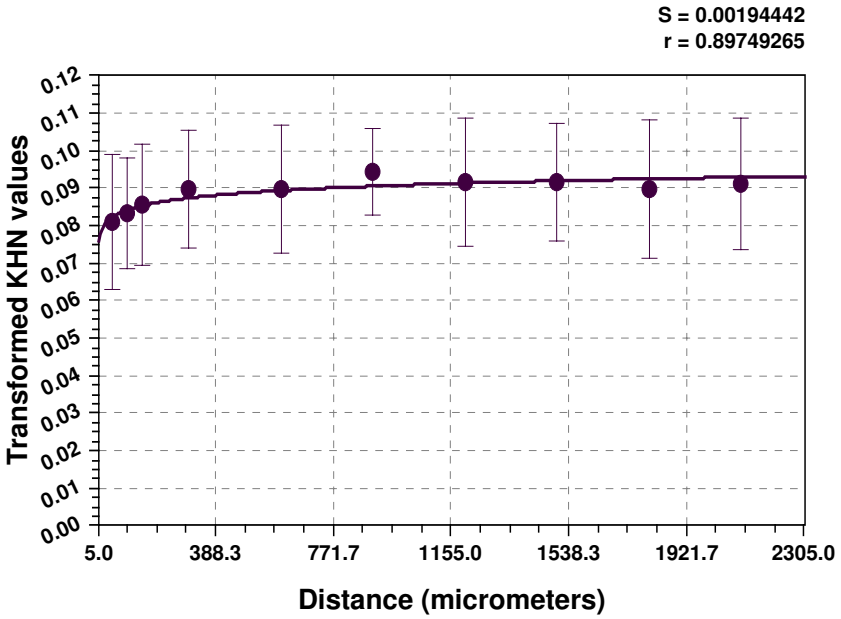
Graph 1. The transformed microhardness values of Ketac-fil were fitted according to a logarithm function (y = 0.0192 + 0.0091lnx).



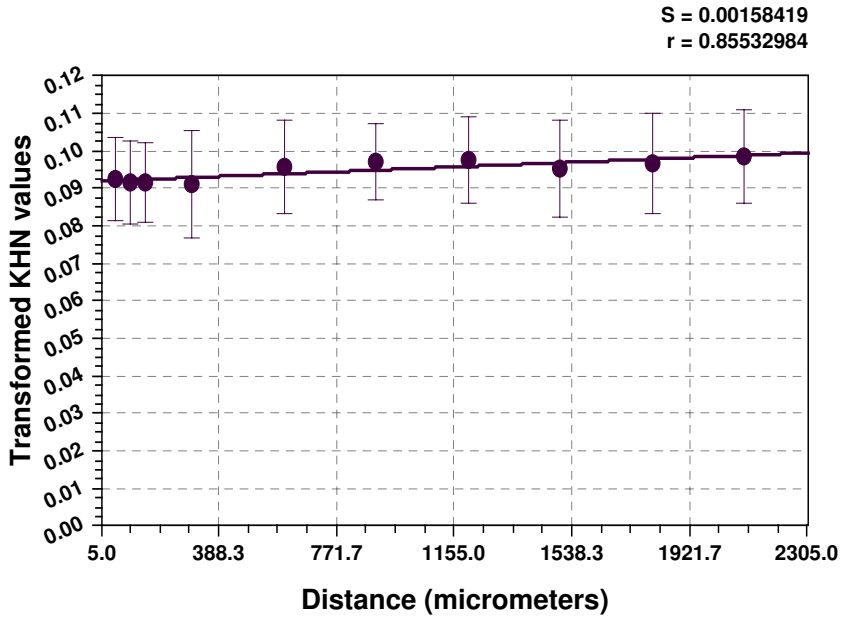
Graph 2. The transformed microhardness values of Fuji II LC were fitted according to a logarithm function (y = 0.0297 + 0.0076lnx).



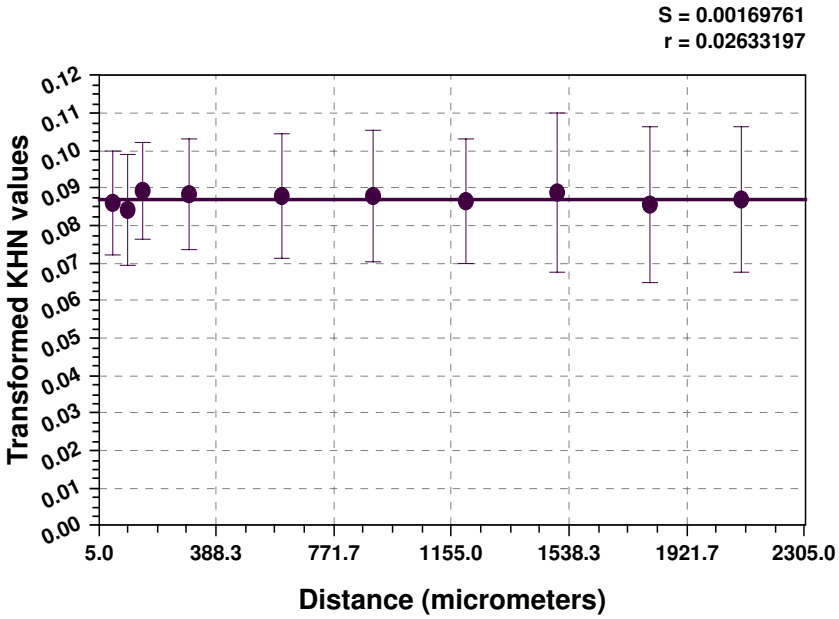
Graph 3. The transformed microhardness values of Surefil were fitted according to a logarithm function (y = 0.0713 + 0.0028lnx).



Graph 4. The transformed microhardness values of Dyract were fitted according to a linear function (y = 0.0919 + 3.28e-006x).



Graph 5. The transformed microhardness values of Z250 were fitted according to a linear function (y = 0.0870 + 5.61e-008x).



LEGENDS TO TABLES, ILLUSTRATIONS AND GRAPHS

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Table 2. Restorative techniques used according to manufacturers' recommendations.

Table 3. Means and standard deviation of transformed (y = 1/x) microhardness values.

Fig 1.

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3. Restoration procedures according to manufacturers' instructions.

4. Restoration (1.5-mm width x 3.0-mm length) and dentin (3.0-mm x 3.0-mm) surface areas left exposed to artificial caries development.

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3.3. CAPÍTULO 3

CARIES RESEARCH, 37(5): 339-344, 2003 (ANEXO 4,5).

CARIES PROGRESSION AND INHIBITION IN HUMAN AND BOVINE ROOT DENTINE IN SITU

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Running title: Caries progression and inhibition in human and bovine dentine

Key Words: Root caries, human dentine, bovine dentine, dentifrice, fluoride

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ABSTRACT

Since the use of bovine instead of human dentine to evaluate cariogenic and anticariogenic substances is not well established, this in situ study was conducted. Eleven volunteers wore palatal acrylic devices, containing 4 dentine slabs (2 human and 2 bovine). Sucrose solution (20%) was dripped over all slabs 4x/day, simulating a cariogenic challenge. Dentifrice slurries, fluoridated or not, were dripped over specified dentine slabs 3x/day to evaluate caries reduction. After 14 days, the biofilm formed on the dentine slabs was collected for microbiological analysis. In dentine, mineral loss (ΔZ) and lesion depth (LD) were determined by cross-sectional microhardness and by polarized light microscopy, respectively. The total streptococci and mutans streptococci counts in the biofilm formed either on human or on bovine slabs, whether treated or not with fluoride dentifrice, was not statistically different. The ΔZ and the LD values of dentine treated with fluoride dentifrice were significantly lower than that treated with non-fluoride dentifrice. The differences in the ΔZ and LD values between the human and bovine dentine were not statistically significant. The results suggest that bovine dentine can be used instead of human to evaluate caries development and inhibition.

INTRODUCTION

In situ models have been used to evaluate the cariogenic and anticariogenic properties of fluoride containing dental products [ten Cate, 1994]. This kind of experiment may be considered as an intermediate stage between in vivo and in vitro studies, allowing the control of clinical conditions related to the development of caries [Manning and Edgar, 1992; Zero, 1995; Sønju Clasen and Øgaard, 1999]. However, some experimental variables of in situ models need to be standardized [Mellberg, 1992], among them the type of dental substrate. Although there is a consensus that the use of human teeth is more relevant to in situ studies [Zero, 1995], bovine teeth have also been used [Manning and Edgar, 1992; ten Cate and van Duinen, 1995; Zero, 1995; Aires et al., 2002]. The advantage of using bovine instead of human teeth is that they are easier to obtain and to manipulate [Mellberg, 1992]. Moreover, they have a relatively more uniform chemical composition, which allows a lower variation in the experimental response of the cariogenic and anticariogenic treatments carried out on the substrate [Mellberg, 1992].

Although bovine enamel is more porous than human enamel, it has been suggested that this discrepancy results only in quantitative and not in qualitative differences in behaviour in an in situ model [Mellberg, 1992; Zero, 1995]. Thus, the use of bovine enamel has been accepted to evaluate the potential of cariogenic and anticariogenic substances [Mellberg, 1992; Zero, 1995]. In contrast, little information has been reported regarding the use of root dentine as an experimental substrate. It is not known whether bovine root dentine has the same behaviour as that of human, either for cariogenic challenges or for treatments with anticariogenic agents - like fluorides - in in situ caries models. However, bovine root dentine has been used [ten Cate and van Duinen, 1995; Aires et al., 2002], considering that the histological and biochemical differences

between the human and bovine dentine may not interfere with the mineral content variation [ten Cate and van Duinen, 1995].

This study was conducted to test whether bovine dentine presents similar behaviour to human dentine in in situ experimental models for root caries progression and inhibition, and also to find out whether the biofilm formed on the dentine substrates is similar in terms of mutans streptococci counts.

MATERIAL AND METHODS

Ethical aspects

This study was approved by the local ethical committee in research (FOP-UNICAMP, process # 017/2002). Eleven adult volunteers took part in this study after signing an informed, written consent (Resolution No. 196 of the National Health Council, Health Ministry, Brasília, DF, 10/03/1996).

Experimental design

The study involved a factorial 2x2 split-mouth design of caries induction by plaque accumulation and sucrose use, performed in one phase of 14 days. The factors under evaluation were: substrate at 2 levels (human and bovine dentine) and treatment at 2 levels (non-fluoride containing and fluoride-containing dentifrice), to simulate free caries progression and inhibited caries progression, respectively. Four experimental groups were obtained (2 substrates x 2 treatments), as follows. Group 1: human dentine and non-fluoride dentifrice; Group 2: human dentine and fluoride dentifrice; Group 3: bovine dentine and non-fluoride dentifrice and Group 4: bovine dentine and fluoride dentifrice. Each group comprised 11 dentine slabs or experimental units (n = 11), randomly assigned to the 11 volunteers, which were considered as statistical blocks. The null hypothesis was that there

was no difference between human and bovine dentine, in caries progression, in caries inhibition or in the composition of the biofilm formed on each tissue. To test this null hypothesis, the following response variables were evaluated: variation in the dentine mineral content (ΔZ) and lesion depth (LD). Additionally, the total streptococci (TS) and mutans streptococci population counts (MS) were analysed in the biofilm formed on the dentine substrates.

Specimen preparation

Root dentine slabs were obtained from human third molars and from bovine incisor teeth, which were sterilized by storage in 10% buffered formalin solution, pH 7, for 7 days [Dominici et al., 2001]. Using two parallel diamond disks separated by a 3 mm spacer, a root slice was cut (Isomet 1000, Buehler) from the cementoenamel junction. The root slices were sectioned mesiodistally and dentine slabs were obtained from the buccal and lingual root surfaces [Aires et al., 2002] (Fig 1A). Thirty slabs (3x3x2 mm) of each type of substrate were obtained. They were flattened and polished, by using 400, 600, 1200 grades of Al_2O_3 papers and polishing cloths with 1 μm diamond paste, respectively (Fig 1B). The slabs were immersed in artificial saliva containing 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.1 M Tris, pH 7.0 [Delbem and Cury, 2002], for 24 h, in order to minimize further ionic changes between the slab and the oral environment [Aires et al., 2002]. The surface microhardness of the 60 dentine slabs was determined (3 indentations in the centre of the slab, 5 g, 5 s). Twenty-two slabs of human and bovine dentine were selected, after having discarding eight slabs of each substrate that presented outlier values of average microhardness.

Palatal device preparation

Acrylic custom-made palatal devices were made with four sites (4x4x4 mm), in which the dental slabs were positioned and fixed with wax (Fig 1C). In order to allow plaque accumulation and to protect it from mechanical disturbance, a plastic mesh was fixed to the acrylic resin, leaving a 1 mm space from the surface of the specimen [Benelli et al., 1993; Cury et al., 2000] (Fig 1D). In groups 1 and 3, the mesh was fixed with a red acrylic resin, to show the volunteers where the non-fluoride dentifrice slurry should be dripped [Cury et al., 2001]. The order in which the experimental units were assigned in the palatal device took into consideration that both groups 1 and 3 should be on the same side of the acrylic device and, consequently, groups 2 and 4 on the opposite side (Fig 1E), to avoid the carry-across effect [Hujoel and DeRouen, 1992] in groups 1 and 3 by the treatment with fluoride dentifrice performed on groups 2 and 4. Within each side of the palatal device, the positions of the specimens were randomly determined.

Intraoral phase

During a one-week lead-in period, and throughout the entire experimental phase, the volunteers brushed their teeth with a non-fluoride silica-based formulation (Kolynos do Brasil Ltd) prepared for this study. Next, all volunteers started to wear the palatal devices.

To provide a cariogenic challenge, the volunteers were instructed to remove the device and to drip 20% sucrose solution onto all blocks 4 x/day (8:00, 11:00, 15:30, 19:00 h). Five minutes later the device was re-inserted in the mouth.

The dentifrice treatment was carried out after the main mealtimes, 3 x/day (7:30, 12:30, 20:00 h), during the volunteers' habitual oral hygiene. The device was removed and slurries (1 : 3 w/v) of non-fluoride dentifrice (silica-based / Kolynos do Brazil Ltd) and fluoride dentifrice (1100 ppm NaF, silica-based / Kolynos do Brazil Ltd) were

dripped onto and remained on the specimens of groups 1 and 3, and 2 and 4, respectively, while the volunteers brushed their teeth with a non-fluoride dentifrice. After that, the device was washed in tap water and re-inserted in the mouth. The volunteers were instructed to wear the intraoral devices the whole time for 14 consecutive days, removing them only to perform the procedures described above and during meals.

Microbiological analysis

On the day 15 of the intraoral phase, 12 and 13 h after the last application of the dentifrice slurries and of the sucrose solution, respectively, the volunteers stopped wearing the intraoral device. The plastic mesh was removed and the biofilm formed on the specimens was collected with sterilized plastic curettes (Fig 1F, 1G). The biofilm was weighed (± 0.01 mg) in pre-weighed microcentrifuge tubes, in which 0.9% NaCl solution was added (1 mL/mg plaque). The tubes were sonicated (Vibra Cell, Sonics & Materials Inc) [Rosalen et al., 1996] and the suspension was serially diluted (1:1, 1:10 and 1:100) with 0.9% NaCl solution. Samples were automatically plated (Spiral plater, DW Scientific Ltd) in duplicate in mitis salivarius agar containing 20% sucrose (MSA), to determine total streptococci, and in mitis salivarius agar plus 0.2 bacitracin/mL (MSB), to determine mutans streptococci [Gold et al., 1973]. The plates were kept for 48 h at 37° C in an anaerobic incubator (IG 150, Jouan) containing 10% CO_2. Representative colonies with typical morphology of mutans streptococci were counted using a colony counter. The total streptococci and mutans streptococci population counts were determined and expressed in CFU per mg wet plaque weight.

Polarized light microscopy and microhardness analysis

The specimens were removed from the device and longitudinally sectioned in their central area, in order to obtain a section of 100 μm (± 10) (Fig 1F, 1H, 1I). These sections were mounted for examination under a polarizing light microscope at 200x

magnification (DMLSP, Leica) after imbibition in deionised water (Fig 1J). Digital images were taken, and the lesion depths were measured at three sites with Image-Pro Plus software (Media Cybernetics). Three measurements were made for each specimen and averaged.

One of the remaining halves of each dentine slab was randomly selected. It was embedded in acrylic resin, the cut surface being exposed, for subsequent flattening and polishing with 400, 600 and 1200 grades of Al_2O_3 papers and polishing cloths with 1 μm diamond paste, respectively. Microhardness was measured using a Knoop indenter with 5 g load for 5 s and a Future-Tech FM microhardness tester coupled to software FM-ARS (Fig 1.K.). Two lanes of eight indentations each were made in duplicate at depths: 10, 20, 30,40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200 and 300 μm from the outer surface of dentine in the central region of the dental slab. The distance between the lanes was 100 μm. Knoop microhardness numbers were obtained and transformed to volume fraction of mineral [Featherstone et al., 1983]. Volume fraction of mineral was plotted against depth for each specimen and the integrated mineral content of the dentine lesion was calculated. A mean value mineral content for depths > 90 μm was used as a measure of the integrated mineral content of inner sound dentine. To compute ΔZ values, the integrated mineral content of the lesion was subtracted from that of sound dentine.

Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked with the Hartley and Shapiro-Wilks tests for the response variables data: mineral loss (ΔZ), lesion depth (μm) and total streptococci (TS) and mutans streptococci population counts (MS). The assumptions were satisfied for the ΔZ and LD data and two-way analysis of variances ($\alpha = 0.05$) was applied. The assumptions were not satisfied for

the bacterial counts, so the two-way Friedman nonparametric test (α = 0.05) was used for these data. The analyses were performed with the SAS System 6.11 software (SAS Institute Inc.).

RESULTS

The microhardness of sound human dentine were higher than sound bovine dentine for either surface (52.3 (5.9) and 39.0 (5.3), $p < 0.0000$) or inner (51.9 (9.5) and 43.4 (12.0), $p = 0.0123$) evaluations.

The ΔZ, LD, TS and MS data for each group are shown in table 1. For the response variables ΔZ and LD, two-way ANOVA revealed no significant interaction between substrate and dentifrice, a non-significant substrate effect and a significant dentifrice effect (Table 2). ΔZ and LD were lower in groups 2 and 3, treated with the fluoride dentifrice (tables 1 and 2). For the TS and MS counts, the two-way Friedman test revealed no significant effect for either substrate or for dentifrice (table 2). The null hypothesis formulated was accepted for all the response variables tested, for both free and inhibited caries progression.

DISCUSSION

The suggestion that caries development in human and in bovine dentine may be considered, in a qualitative analysis, as a similar process [Mellberg, 1992; Zero, 1995] was confirmed by this study, since the differences in dentine mineral loss and lesion depth between both bovine and human dentine substrate were not statistically significant. Moreover, the inhibition of root caries, by fluoride dentifrice application, was similar to both tested substrates. Nevertheless, these results does not imply that there are no structural

differences between them, since the microhardness values of sound human root dentine were higher than sound bovine dentin, for either surface or inner analysis.

Fluoride-containing dentifrice has proven to be capable of reducing root caries development in vitro [Mukai et al., 2001], in situ [Nyvad et al., 1997] and in vivo [Jensen and Kohout, 1988; Baysan et al., 2001]. This anticaries capacity has been related to physicochemical effects, by inhibiting demineralisation and enhancing remineralisation processess [Featherstone, 1994], and also to antibacterial effects, by inhibiting the critical metabolic processess of mutans streptococci and by preventing the development of favourable low pH environments for cariogenic bacteria in the biofilm [Bradshaw et al., 2002]. In the current study, although the fluoride dentifrice inhibited the caries development, both in human and bovine root dentine, it was not able to reduce TS and MS counts by the fluoride dentifrice application. This suggests that the inhibition of demineralisation and enhancement of remineralisation [Featherstone, 1994] were more important caries-preventive factors than the absolute reduction of these cariogenic microrganisms.

Different methods have been used to assess the caries progression on root dentine. Among them, microhardness is the only one that considers alterations on both organic and inorganic content of dentine. However, this method is limited by the shrinkage after the drying necessary for microhardness measurement. This could have introduced error into the dentine mineral content in the lesion. However, even causing undesirable effects, drying of specimens did not influence the results, since the lack of differences in the caries development between human and bovine dentine was also confirmed by polarized light microscopy, another recognized method that allow the specimens to be kept in wet conditions during evaluation [Wefel, 1995].

It is important to consider that the sterilization method by aldehyde solutions, such as formaldehyde - that was used in this study - or glutaraldehyde may stabilize collagen by fixing the protein [Haller et al., 1993; DeWald, 1997], possibly altering the demineralising and remineralising behaviours in the root caries processes [Arends et al., 1989; Boonstra et al., 1993; Kawasaki and Featherstone, 1997]. Arends et al. [1989] suggested that this fixative effect is limited to the dentine surface, but this has not yet been proved and further work is required.

An important aspect that should be considered prior to the use of bovine teeth in in situ studies is the occurrence of an infectious disease called spongiform encephalopathy, or mad cow disease [Institute of Food Science and Technology, 2001]. This disease attacks bovines and produces a progressive neuro-degenerative lesion, by means of an infectious prion [Prusiner, 1982]. This prion cannot be eliminated by irradiation, heat or chemical sterilization methods [Taylor et al., 1994]. Although there are no cases reported in the literature of the transmission of this disease in humans, the use of bovine teeth in countries where spongiform encephalopathy has been diagnosed should be carefully analysed. Until now, there has been no effective treatment for this disease [Institute of Food Science and Technology, 2001].

As a conclusion, bovine dentine substrate can be used as a substitute for human dentine substrate in in situ caries models for studies of cariogenic and anticariogenic agents.

ACKNOWLEDGMENTS

This manuscript is part of a thesis submitted by the first author to the Faculty of Dentistry of Piracicaba, University of Campinas (UNICAMP), in partial fulfilment of the

requirements for the Doctoral degree in Clinical Dentistry (Restorative Dentistry). The authors would like to express their gratitude to all volunteers that took part in this study. This study was supported by FAPESP (Process # 00/14278-0).

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TABLES

Table 1. Mean ∆Z, LD, TS and MS (standard deviation in parenthesis) for the experimental groups (n = 11).

Groups	∆Z (vol % min.μm)	LD (μm)	TS (10^6 CFU/mg)	MS (10^6 CFU/mg)
1: Human / non fluoride	243.4 (173.3)	35.8 (23.5)	17.6 (6.2)	2.0 (3.6)
2: Human / fluoride	55.6 (77.2)	7.7 (10.5)	20.4 (6.5)	1.1 (1.7)
3: Bovine / non fluoride	211.7 (113.0)	31.1 (17.6)	19.7 (5.9)	0.6 (1.0)
4: Bovine / fluoride	2.8 (107.6)	8.3 (8.0)	17.6 (7.6)	1.0 (1.7)

∆Z: mineral loss, LD: lesion depth, TS: total streptococci, MS: mutans streptococci, CFU: colony forming unit.

Table 2. P-values obtained for each response variable tested according to the factors under study.

Factors	∆Z*	LD*	TS**	MS**
Substrate x Dentifrice***	0.7262	0.3351	---	---
Substrate	0.3600	0.4528	0.1797	0.3710
Dentifrice	0.0001	0.0004	0.6547	1.0000

∆Z: mineral loss, LD: lesion depth, TS: total streptococci count, MS: mutans streptococci population count.
Significance level adopted: 0.05.
* Two-way ANOVA.
** Friedman non-parametric test.
*** Interaction of interest.

ILLUSTRATION

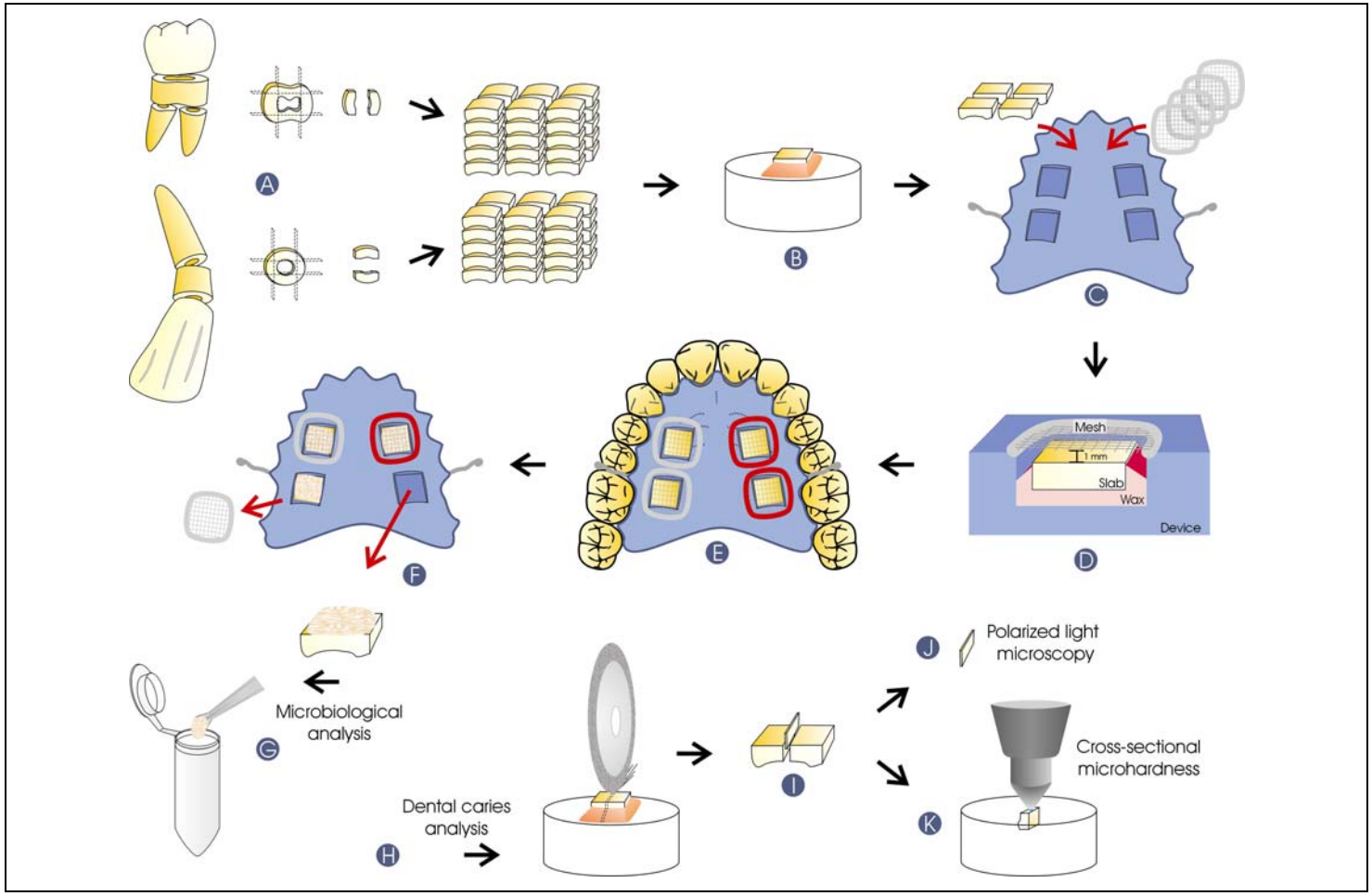

Fig 1. A. Human and bovine dentine slabs obtainment; B. Flattened and polished slabs; C. Palatal device preparation; D. Scheme of the slab position in the palatal device. Note that 1 mm was left between the mesh and the slab surface; E. Intraoral phase; F. After 14 days, the mesh was removed; G. The biofilm was collected for microbiological analysis; H,I. The dentin slab was sectioned in the central area; J.A dentin section was analysed by polarized light microscopy; K. One of the remaining halves was randomly assigned to cross-sectional microhardness measurements.

LEGENDS

Fig 1.

A. Human and bovine dentine slabs obtainment.

B. Flattened and polished slabs.

C. Palatal device preparation.

D. Scheme of the slab position in the palatal device. Note that 1 mm was left between the mesh and the slab surface.

E. Intraoral phase.

F. After 14 days, the mesh was removed.

G. The biofilm was collected for microbiological analysis.

H, I. The dentin slab was sectioned in the central area.

J. A dentin section was analysed by polarized light microscopy.

K. One of the remaining halves was randomly assigned to cross-sectional microhardness measurements.

3.4. CAPÍTULO 4

SUBMETIDO PARA PUBLICAÇÃO / JOURNAL OF DENTAL RESEARCH (ANEXO 6,7).

INFLUENCE OF FLUORIDE-RELEASING RESTORATIVE MATERIAL ON ROOT DENTIN SECONDARY CARIES IN SITU

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Short title: Fluoride-releasing restorative material and secondary caries

Key words: Root caries, root dentine, glass-ionomer cement

Number of words in: abstract: 150; abstract + text: 2500; number of tables and figures: 2; number of cited references: 29.

Based on a thesis submitted by the first author to the Faculty of Dentistry of Piracicaba, University of Campinas, SP, Brazil, in partial fulfillment of the requirements for the PhD degree.

ABSTRACT

This study tested the hypothesis that fluoride-releasing restorations, either aged or un-aged, do not prevent secondary root caries, when fluoride dentifrice is frequently used. Sixteen volunteers wore palatal appliances in 2 phases of 14 days, according to a 2x2 *cross-over* design. In each phase the appliance was loaded with bovine root dentine slabs restored with either glass-ionomer or resin composite, either aged or un-aged. Specimens were exposed to cariogenic challenge 4x/day and to fluoridated dentifrice 3x/day. The fluoride content in the biofilm (FB) formed on slabs and the mineral loss (ΔZ) around the restorations were analyzed. No differences were found between restorative materials regarding the FB and the ΔZ, for both aged ($p=0.792$ and $p=0.645$, respectively) and un-aged ($p=1$ and $p=0.278$, respectively) groups. Under the cariogenic and fluoride dentifrice exposure conditions of this study, the glass-ionomer restoration either aged or un-aged did not provide additional protection against secondary root caries.

INTRODUCTION

Fluoride has shown an important role in the control of root caries, since it may interfere in related physicochemical (Featherstone, 1994) and microbiological (Bradshaw *et al.*, 2002) processes, not only reducing the caries progress rate but also allowing the arrestment of active lesions (Nyvad and Fejerskov, 1986). Common and affordable sources of fluoride, such as dentifrices may reduce root caries development, as previously showed *in situ* (Hara *et al.*, 2003) and *in vivo* (Jensen and Kohout, 1988). Possibly, this effect may be extended to secondary root caries as well, since this entity has been considered as primary caries adjacent to restorations (Thylstrup, 1998; Mjör and Toffenetti, 2000).

Intending to provide fluoride to the specific site at risk of secondary caries occurrence, fluoride-releasing restorative materials were developed. Their cariostatic effect on root dentine has been reported in *in vitro* studies (Tam *et al.*, 1997; Dyonisopoulos *et al.*, 1998; Pereira *et al.*, 1998). However, these findings were not evidenced in a systematic review of clinical studies of secondary caries around glass-ionomer cement restorations (Randall and Wilson, 1999). This suggests that fluoride-containing restorations do not prevent secondary caries. Intending to clarify this controversy, it was hypothesized in this study that fluoride coming from another sources, like dentifrice, could replace that released by the restoration; or, that the cariostatic potential of restorations could be reduced as their fluoride releasing rate declines through the aging process (Carvalho and Cury, 1999).

In the present study, these above-mentioned hypotheses were tested in well-controlled clinical conditions, provided by *in situ* caries model (Zero, 1995). A previously described root caries model (Hara *et al.*, 2003) was used including fluoridated dentifrice exposures and specimens with restorations.

MATERIAL AND METHODS

Ethical aspects

The research protocol was approved by the local ethical committee in research (FOP-UNICAMP, process # 041/2001). Sixteen adult volunteers took part in this study after signing an informed, written consent (Resolution No. 196 of the National Health Council, Health Ministry, Brasília, DF, 10/03/1996).

Experimental design

The study was conducted according to a factorial 2×2 *cross-over* design, performed in two phases of 14 days. The factors under evaluation were restorative material, at 2 levels: glass-ionomer cement (GIC) and resin composite (RC); and age of restoration, at 2 levels: aged (A) and un-aged (U), resulting in 4 experimental groups: 1-GIC/A, 2-GIC/U, 3-RC/A and 4-RC/U. Each group comprised 32 restored dentine slabs in duplicates or 16 experimental units (n = 16). They were randomly assigned to the 16 volunteers, which were considered as experimental blocks. In phase 1, eight volunteers wore appliances with specimens of groups 1 and 2 and eight with groups 3 and 4, avoiding a possible *carry-across* effect (Hujoel and DeRouen, 1992) of GIC on RC. In phase 2, volunteers that had worn appliances with specimens of groups 1 and 2, wore appliances loaded with specimens of groups 3 and 4 and *vice-versa*. The main responses variables were fluoride concentration in the biofilm formed over the slabs (FB: μg F/g biofilm) and mineral loss (ΔZ: vol % min x μm). The null hypotheses tested were that there were no differences between the GIC and RC, either aged or un-aged, with regards to their ΔZ and FB, when submitted to cariogenic conditions in the presence of regular fluoridated dentifrice exposures.

Specimen preparation

Two hundred and fifty six bovine root dentine slabs (5 x 5 x 2 mm) were cut, stored in 10 % buffered formalin solution for 1 week and grinded as described by Hara *et al.* (2003). Box-shaped cavities (3.3 x 1.5 x 1 mm) were prepared on the center of each slab, with diamond burs at high-speed rotation and under water/air spray cooling.

A hundred twenty eight slabs in duplicates (or 64 experimental units) were randomly assigned to each phase. They were restored with either GIC (Ketac-fil plus, Espe GmbH, Seefeld, Germany) or RC (Filtek Z 250/Single Bond, 3M Dental Products, St Paul, MN, USA), following the manufacturers' instructions, and stored for 24 h in 100 % relative humidity, at 37 ^{o}C $\pm$ 1. To remove any possible excess of restorative material, specimens were ground with # 1200 grit Al_2O_3 abrasive papers, under water-cooling. Subsequently, specimens were polished with 1 μm diamond suspension on cloths and sonicated for 1 min in cleaning solution.

Specimens aging and palatal device preparation

Specimens of groups 1 and 3 were prepared prior than those of groups 2 and 4, and they were individually immersed in artificial saliva (10 mL/specimen; 1.5 mM Ca, 0.9 mM P, 0.1 M Tris buffer, pH 7.0), at 37 ^{o}C $\pm$ 1. The solution was changed daily for 14 consecutive days, in order to simulate an aging process (Hsu *et al.*, 1998). Fluoride released in the artificial saliva was measured (Hayacibara *et al.*, 2004) and values of cumulative release of fluoride (CF: μg F/cm^2) were computed. A dentine surface area (5 x 1.75 mm) adjacent to the restoration was covered with an acid-resistant varnish to act as a control (Figure).

Acrylic custom-made palatal devices were built with four sites (6 x 6 x 4 mm), in which the dental slabs were positioned and fixed with resin composite. In order to allow

plaque accumulation and to protect it from mechanical disturbance, a plastic mesh was fixed to the acrylic resin, leaving a 1 mm space from the surface of the specimen (Hara *et al.*, 2003).

Intraoral phase

Volunteers followed a one-week *lead-in* period before inserting the palatal devices. During this period and throughout the experimental phase, they brushed their teeth with a silica-based dentifrice (Sorriso Fresh Mint, Kolynos do Brasil Ltd), containing 1100 μg F/g (w/w) as NaF.

The cariogenic challenge to the specimens was provided by dripping a solution of 20% sucrose onto all blocks 4 times a day (8:00, 11:00, 15:30, 19:00 h) that was kept in contact for 5 min (Hara *et al.*, 2003). Toothbrushing with the fluoride dentifrice was performed after the main mealtimes, 3 times a day (7:30, 12:30, 20:00 h). Volunteers were instructed to use a pea-size amount of dentifrice and to start brushing the buccal surface of upper teeth with the appliance still in the mouth. After the slurry of dentifrice and saliva reached the plastic mesh over the specimens (approximately 10 s), the appliance was removed and kept without rinse until the volunteers finished their habitual oral hygiene. After that, the device was washed in tap water, removing all dentifrice/saliva slurry, and re-inserted in the mouth. Volunteers were instructed to wear the intraoral devices the whole intraoral phase, except during brushing procedures and meals. At these times, the devices were kept moist in boxes that were provided.

Fluoride measurement in the dental biofilm

After each phase, 12 h after the last brushing procedure, the plastic meshes of the appliances were removed and the biofilm formed on specimens was collected with plastic curettes and frozen. The biofilm was dried in a vacuum atmosphere in the presence of P_2O_5, for 24 h. Then, the dry weight of each sample was determined in pre-weighed

tubes before 0.5 M HCL was added (0.25 mL/mg of biofilm). After extraction for 3 h at room temperature under constant agitation, the same volume of TISAB II, pH 5.0 (containing 20 g NaOH/L), was added to the tube as a buffer (Benelli *et al.*, 1993; Cury *et al.*, 2000). The samples were centrifuged (11,000 *g*) for 1 min and the amount of acid-soluble F in the supernatant was determined using an ion-selective electrode Orion 96-09 and an ion analyzer Orion EA-940.

Transverse microradiography analysis

The specimens were removed from the palatal device and sectioned through the center of the restoration, in order to obtain sections of 160 μm ± 20. The sections were x-rayed together with an aluminum stepwedge, at 20 kV and 30 mA for 65 min. Microradiographic plates were processed and the radiographic images were taken and analyzed with dedicated computer software (TMR Version 1.26, Inspektor Research Systems BV, The Netherlands). ΔZ measurements were made in three zones (50 μm wide and 300 μm deep) 50, 150 and 300 μm apart from the restoration in both exposed and control sides (Figure). Average values of ΔZ in each side were computed and the net ΔZ value ($\Delta Z_{net} = \Delta Z_{exposed} - \Delta Z_{control}$) was obtained and submitted to the statistical analysis.

Statistical analysis

The cumulative fluoride released during the aging process was evaluated by the Student-t test. The presence of *carry-over* and phase effects in the responses was checked. The FB and ΔZ were tested independently in both aged (1 and 3) and un-aged (2 and 4) groups. Comparisons between the aging effects in the variable responses were made to either GIC or RC restorations on the phase 1 data of this study. Statistical analyses were performed by the Wilcoxon non-parametric test, with 5% of significance level.

RESULTS

During the aging process, group 1 released significantly higher amounts of fluoride than group 3 ($p < 0.01$) (Table). There were no *carry-over* and phase effects to both aged and un-aged restorations ($p < 0.05$). No differences were found in the FB between GIC and RC, either aged ($W = 28$, $p = 0.792$) or un-aged ($W = 30$, $p = 1$), as well as in the ΔZ between GIC and RC, either aged ($W = 63$, $p = 0.645$) or un-aged ($W = 57$, $p = 0.278$) (Table). No significant differences were found when comparisons were done between the ΔZ of aged and un-aged restorations to either the GIC ($W = 64$, $p = 0.798$) or RC ($W = 65$, $p = 0.729$). All null hypotheses formulated were accepted.

DISCUSSION

The *in situ* caries model used in the current study, based on biofilm accumulation and sucrose exposure, was previously reported to be cariogenic to bovine root dentine (Aires *et al*., 2002; Hara *et al*., 2003). Our experimental model included the use of fluoride-containing dentifrice, since most of the marketed dentifrices have been reported to be fluoride containing (Donly and Nelson, 1997). The contact of the dentifrice slurry with the biofilm/specimen during the brushing procedure modeled the clinical situation of fluoride (dentifrice) exposure to surfaces at risk of caries (Dugall *et al*., 2001). Thus, the current *in situ* model was considered suitable for the test of the proposed hypotheses.

No differences were found between the net mineral losses in root dentin around restorations with the tested materials, accepting the stated hypothesis that the glass-ionomer cement might not be necessary to prevent secondary caries in root dentine, when fluoride dentifrice is frequently used. This suggests an effect of the dentifrice in

secondary caries. Although it has already been shown that the use of fluoridated dentifrice can inhibit root caries (Jensen and Kohout, 1988), no clinical evidences of this effect on secondary root caries prevention have been previously reported. The current results seem to corroborate earlier *in vitro* studies (Donly and Kerber, 1999; Hara *et al*., 2002), where a significant effect of fluoride-containing dentifrice on secondary root caries inhibition was found. However, these *in vitro* studies have not considered the relevance of the dental biofilm in cariogenic and fluoride-retention processes, as it was done in the present study.

The fluoride concentration found in the dental biofilm was comparable with a previous study that used similar cariogenic challenge and dentifrices (Paes Leme *et al*., 2004), and was not significantly influenced by the restorative materials tested. Exposure to fluoride may cause an increase in the fluoride levels of saliva, soft tissues and biofilm (Paes Leme *et al*., 2004; Hossain *et al*., 2003), creating a fluoride reservoir for further cariogenic attacks (Duckworth and Morgan, 1991). Furthermore, fluoride has shown to reduce the cariogenicity of biofilm formed *in vitro* (Bradshaw *et al*., 2002). The obvious advantage of using topical fluoride delivery vehicles, like dentifrices, is that in addition to preventing caries around the margins of restorations, they can reach any other site in the mouth at risk for caries.

The hypothesis that the restoration aging process could be related to the absence of the cariostatic effect was not proven to be true. Significant amount of fluoride was released by the GIC during this process, however it did not influence either the ΔZ or the FB. It seems that the amount of fluoride released by the material, even considering periods of higher releasing rate during the first days after the restorative procedures (Carvalho and Cury, 1999), has little or no influence in secondary root caries prevention, when fluoride dentifrice is used with a frequency of 3 times a day. Although this study was not specifically designed to test the rechargeability of the GIC restorations, it was expected

to occur during the dentifrice exposure (Freedman and Diefenderfer, 2003). However, based on the current results it is unclear if this reported GIC property might be relevant to secondary caries prevention.

Subjects that participated in our study were not considered to be of high-risk for caries, since they presented normal salivary flow, normal fluoride exposure and no current caries activity, except for the specimens in the micro-environment created by the *in situ* model. Clinical evaluations of xerostomic patients have shown secondary caries inhibition around both fluoridated and non-fluoridated cervical restorations when fluoride gel was daily used (Wood *et al.*, 1993; McComb *et al.*, 2002). These data and evidence from a *in vitro* study (Donly and Kerber, 1999) and a systematic review (Randall and Wilson, 1999) have suggested that glass-ionomer cement restorations may not be important to prevent secondary root caries, depending on the balance between the risk for caries and the fluoride exposure. Accordingly, the choice of the restorative material for root lesions may not be guided by its supposed cariostatic potential, when the individual requirement of fluoride is fulfilled by another sources. Instead, it should consider the mechanical and adhesive properties of the restorative as well as its surface and esthetics characteristics.

It can be concluded that in this experiment glass-ionomer restoration, either aged or un-aged, provided no additional effect in preventing secondary root caries in the presence of regular exposures to fluoridated dentifrice.

ACKNOWLEDGEMENTS

To Mariza Soares for the technical assistance. Supported by the State of São Paulo Research Foundation (FAPESP # 00/14278-0) and Coordination of Training of Higher Education Graduate (CAPES # BEX 2443/02-3), Brazil.

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TABLES

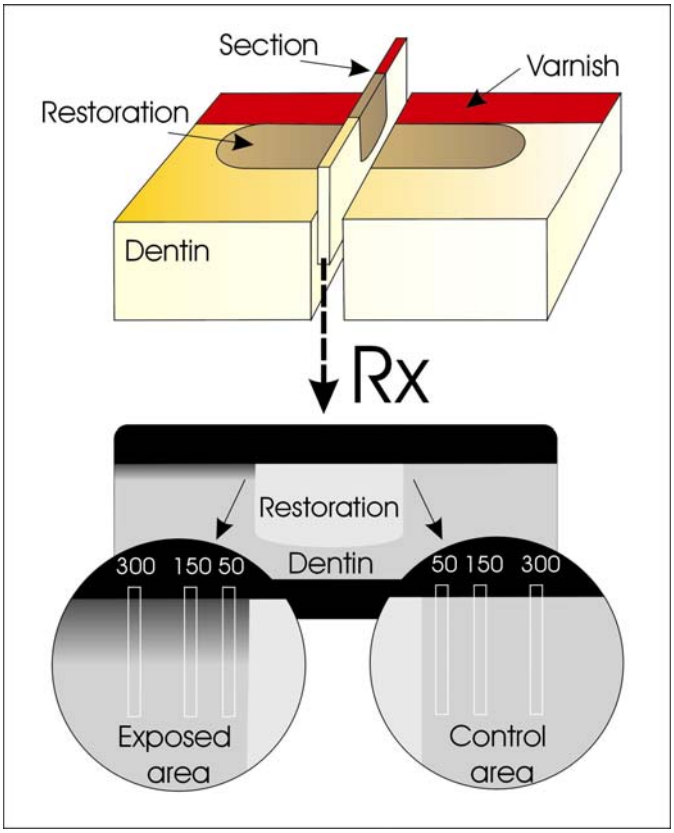
Table 1. Summary of results (mean (SD); median) and statistical analysis.

Groups	Cumulative F[a]		Fluoride in the biofilm[b]			Mineral loss[c]		
	Mean	*t*-test	Mean	Median	W[d]	Mean	Median	W
1. GIC/ aged	34.67 (15.70)	SIG[e]	311.88 (349.34)	190.32	NS[f]	128.73 (191.31)	50.69	NS
3. RC/ aged	-1.31 (3.49)		278.11 (166.27)	269.43		151.32 (268.62)	21.18	
2. GIC/ un-aged	---	---	254.19 (241.27)	136.71	NS	123.81 (144.55)	116.33	NS
4. RC/ un-aged	---		240.01 (204.44)	199.59		155.26 (287.45)	24.38	

[a] μg F/cm^2; [b] μg F/g of dry biofilm; [c] %vol min.μm; [d] Wilcoxon non-parametric test; [e] statistically significant ($\alpha = 0.05$); [f] statistically non-significant ($\alpha = 0.05$)

FIGURES

Figure 1. Section obtaining from the specimen and transverse microradiography analysis.



FIGURES LEGENDS

Figure 1. Section obtaining from the specimen and transverse microradiography analysis.

4. DISCUSSÃO GERAL

Os diferentes resultados obtidos na análise *in vitro* do efeito cariostático dos materiais restauradores fluoretados - **Capítulos 1 e 2** - parecem estar relacionados à liberação de fluoretos durante o desafio cariogênico. O CIV e o IVMR, cujas capacidades de liberar fluoretos têm sido descritas (Eichmiller & Marjenhoff, 1998; Carvalho & Cury, 1999; Karantakis *et al.*, 2000; Vermeersch *et al.*, 2001), inibiram a progressão de cárie artificial em dentina radicular em considerável distância a partir das margens das restaurações. Por outro lado, materiais como a RCF e a RCMP, que têm apresentado liberação de fluoretos em concentrações relativamente menores (Eichmiller & Marjenhoff, 1998; Carvalho & Cury, 1999; Karantakis *et al.*, 2000; Vermeersch *et al.*, 2001), não foram capazes de evidenciar tal efeito. Este resultado contradiz estudos anteriores que atribuíram algum efeito cariostático à RCF (Dionysopoulos *et al.*, 1998; Torii *et al.*, 2001) e principalmente à RCMP (Dionysopoulos *et al.*, 1998; Donly & Grandgenett, 1998; Millar *et al.*, 1998). Possivelmente, tal diferença esteja relacionada às marcas comerciais avaliadas e também aos procedimentos experimentais, devido ao emprego de diferentes modelos de cárie artificial e diferentes métodos para quantificação da lesão. Os modelos de cárie *in vitro* utilizados nos **Capítulos 1 e 2**, fundamentados em ciclos de des-remineralização, foram escolhidos por reproduzirem a dinâmica de formação de cárie e por serem de execução relativamente rápida e simples (Featherstone, 1996).

Determinados SAFs foram capazes de liberar fluoretos em quantidades similares ao CIV, ao serem testados com dimensões e volumes similares. Entretanto, ao serem associados a um material restaurador simulando condições clínicas, nenhum dos SAFs foi capaz de inibir o desenvolvimento de cárie artificial. Provavelmente, a importância dos SAFs na inibição de cárie resume-se à proteção da zona de interdifusão

resina-dentina - denominada “camada híbrida” (Nakabayashi *et al.*, 1991) - frente à infiltração marginal e também à desmineralização, evitando o desenvolvimento de lesões de parede (Kidd, 2001). Porém, ao se considerar que lesões de parede relacionam-se principalmente com restaurações não adesivas e tem pouco ou nenhum envolvimento com o desenvolvimento da cárie secundária natural (Thylstrup, 1998), sugere-se que nenhum efeito cariostático significativo possa ser atribuído aos SAFs, em condições clínicas.

Embora os resultados obtidos no **Capítulo 2** demonstrem o potencial de determinados materiais restauradores para inibir a cárie radicular secundária *in vitro*, a reprodução clínica dessa propriedade cariostática tem sido questionada (Randall & Wilson, 1999). Com o intuito de conferir relevância clínica para a análise do efeito cariostático de materiais restauradores foi desenvolvido um modelo de cárie radicular *in situ* - **Capítulo 3** - capaz de avaliar a desmineralização e remineralização da dentina humana e bovina em condições próximas às encontradas clinicamente. O modelo de cárie proposto, com base no acúmulo de biofilme e na aplicação de sacarose, demonstrou ser suficientemente cariogênico para produzir lesões de cárie mensuráveis. Além disso, o uso de soluções fluoretadas nesse modelo produziu respostas significativas quanto à remineralização da cárie radicular. O comportamento semelhante entre os substratos humano e bovino frente às condições impostas pelo experimento confirmou a possibilidade do uso da dentina radicular bovina para o estudo da cárie radicular.

Modelos de cárie *in situ* reproduzem a dinâmica envolvida no desenvolvimento da cárie permitindo analisar de maneira controlada o efeito de tratamentos na prevenção ou inibição das lesões cariosas. Entretanto, limitações existem quanto ao estudo de diversos fatores e níveis experimentais (Jones & Kenward, 1989). Optou-se, então, pela

análise apenas do CIV no **Capítulo 4** devido à melhor efetividade deste material na inibição da cárie radicular secundária *in vitro*, conforme observado nos **Capítulos 1 e 2** e em outros estudos relatados na literatura (Dionysopoulos *et al*., 1998; Pereira *et al*., 1998b; Hara *et al*., 2002). A projeção do desempenho obtido pelo CIV para outros materiais restauradores deve ser feita de maneira indireta. Os resultados observados no **Capítulo 4** reforçam a hipótese de que as diferenças entre estudos *in vitro* e *in vivo* para análise do efeito cariostático de materiais restauradores podem estar relacionadas ao uso constante de fontes de fluoretos, tais como o dentifrício, que levaria a formação de reservatórios de íons flúor tornando desnecessário o uso de materiais restauradores fluoretados (Duckworth & Morgan, 1991; Shellis & Duckworth, 1994). Embora a freqüência ótima para a utilização de fluoretos em função do risco de cárie radicular não tenha sido ainda determinada, optou-se pela exposição dos espécimes ao dentifrício fluoretado por 3 x/dia, considerando ser esta uma freqüência passível de ser reproduzida clinicamente.

Sob as condições cariogênicas e de uso de fluoretos adotados no experimento não houve proteção adicional contra a cárie radicular secundária proporcionada pelo uso de um material restaurador fluoretado. Resultados semelhantes foram encontrados clinicamente ao se observar que a aplicação tópica diária de gel fluoretado inibiu o desenvolvimento de cárie radicular ao redor de restaurações, fluoretadas ou não, em pacientes xerostômicos (Wood *et al.*, 1993; McComb *et al.*, 2002). Sugere-se que a indicação de materiais restauradores fluoretados pode não ser relevante, se existir um equilíbrio no balanço entre o risco individual de cárie radicular secundária e a exposição a fluoretos, independentemente das fontes de íons flúor envolvidas. Embora os materiais restauradores fluoretados contribuam para o alcance desse equilíbrio, isso ocorrerá apenas nas adjacências da restauração, numa extensão aproximada de até 0,3 mm,

conforme observado no **Capítulo 2**. Por outro lado, o uso de fontes de íons flúor mais abrangentes, tais como o dentifrício, soluções de bochecho e géis, em concentrações e freqüência adequadas ao risco individual de cárie, permitirá a prevenção não apenas da cárie radicular secundária mas também de qualquer outro processo carioso existente. Esses dados e os resultados do **Capítulo 4** permitem questionar a relevância do material restaurador como fonte de fluoretos estrategicamente posicionada adjacente ao sítio de desenvolvimento da cárie secundária.

Conseqüentemente, a escolha do material restaurador para lesões radiculares deve considerar o risco de cárie do paciente, o grau de exposição deste a outras fontes de fluoretos e as particularidades de cada material restaurador. Para indivíduos com exposição de fluoretos compatível com o risco de cárie (Cury, 2001) pode-se priorizar as propriedades físico-mecânicas e adesivas do material restaurador, assim como suas características superficiais e estéticas, em detrimento do potencial cariostático.

5. CONCLUSÃO GERAL

Conclui-se, com base nos estudos realizados nesta tese, que:

1. sistemas adesivos fluoretados, embora capazes de liberar íons flúor, não apresentam potencial anticariogênico com relação à dentina radicular, em condições *in vitro*;
2. a extensão *in vitro* do efeito cariostático do CIV e do IVMR foi de aproximadamente 0,30 e 0,15 mm, respectivamente, a partir das margens da restauração, sendo que nenhum efeito cariostático foi observado para a RCMP e a RCF;
3. o modelo *in situ* de cárie em dentina radicular bovina, fundamentado no acúmulo de biofilme e uso de sacarose, demonstrou ser adequado para estudo da progressão e inibição - por fluoreto - da cárie radicular;
4. o CIV parece não influenciar o desenvolvimento da cárie radicular secundária, se dentifrício fluoretado for utilizado freqüentemente.

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ANEXO 1

DELIBERAÇÃO CCPG – 001/98

Dispõe a respeito do formato das teses de Mestrado
e de Doutorado aprovadas pela UNICAMP

Tendo em vista a possibilidade, segundo parecer PG Nº 1985/96, das teses de Mestrado e Doutorado terem um formato alternativo àquele já bem estabelecido, a CCPG resolve:

Artigo 1º - Todas as teses de mestrado e de doutorado da UNICAMP terão o seguinte formato padrão:

I) Capa com formato único, dando visibilidade ao nível (mestrado e doutorado), e à Universidade.
II) Primeira folha interna dando visibilidade ao nível (mestrado ou doutorado), à Universidade, à Unidade em foi defendida e à banca examinadora, ressaltando o nome do orientador e co-orientadores. No seu verso deve constar a ficha catalográfica.
III) Segunda folha interna onde conste o resumo em português e o Abstract em inglês.
IV) Introdução Geral.
V) Capítulo.
VI) Conclusão geral.
VII) Referências Bibliográficas.
VIII) Apêndices (se necessários).

Artigo 2º - A critério do orientador, os Capítulos e os Apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

Parágrafo único – Os veículos de divulgação deverão ser expressamente indicados.

Artigo 3º - A PRPG providenciará o projeto gráfico das capas bem como a impressão de um número de exemplares, da versão final da tese a ser homologada.

Artigo 4º - Fica revogada a resolução CCPG 17/97.

ANEXO 2

European Journal of Oral Sciences

Professor Jorma Tenovuo, EDITOR
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Prof. Jaime A. Cury
Av. Limeira 901
Piracicaba, SP
13414-900 BRAZIL

Turku 2004-08-02

Dear Dr. Cury,

Thank you for submitting your article entitled *Fluoride release and secondary caries inhibition by adhesive systems on root dentine* to the **European Journal of Oral Sciences.**

Please refer to **manuscript number 1902** on all further correspondence.

Your manuscript has been forwarded to the reviewers and you will be contacted again as soon as the necessary information for an editorial decision has been obtained.

Sincerely yours,
Kind regards

Jorma Tenovuo
Editor

ANEXO 3

©Operative Dentistry, 2002, 27, 480-487

Extent of the Cariostatic Effect on Root Dentin Provided by Fluoride-Containing Restorative Materials

AT Hara • CP Turssi
MC Serra • MCS Nogueira

Clinical Relevance

The extent to which fluoride is effective around a glass ionomer cement and a resin-modified glass ionomer are estimated to be about 0.3 and 0.15 mm, respectively, in root dentin. This could be important for reducing secondary root caries development.

SUMMARY

This study evaluated the extent of the cariostatic effect on root dentin provided by four fluoride-containing restorative systems: Ketac-Fil/ESPE [Ke], Fuji II LC *Improved*/GC Corp [Fj], Dyract AP/Dentsply [Dy] and SureFil/Dentsply [Su], and one without fluoride: Z250/3M [control]. Ninety-five bovine root dentin fragments (5.0 x 6.0 mm) were obtained, embedded in polyester resin and planed. Cavities (1.5 x 3.5 x 1.0 mm) were made and restored by the five restorative systems (n=19) in a randomized complete block design according to the manufacturers' instructions. After 24 hours, the dentin/restoration surface was polished. The restoration surface and an adjacent area of 3.0 x 3.0 mm were demarcated and submitted to a pH-cycling model. Dentin surface Knoop microhardness values were obtained (5.0-g, 5.0-s) for 10 distances: 50, 100, 150, 300, 600, 900, 1200, 1500, 1800 and 2100 µm from the margin of the restoration. The dentin microhardness means for each restorative material at each distance was considered by the ANOVA multi-factor split-plot method. The interaction between the restorative system and distance was statistically significant ($p<0.05$). The Tukey test and the regression analysis showed that the means of [Ke] and [Fj] were similar up to 300 µm, the [Ke] means being higher than the [control] at distances 50, 100, 150 and 300 µm. The [Fj] means were higher than the [control] at distances 50, 100 and 150 µm. The microhardness means of [Dy] and [Su] were not statistically different from the [control] and remained steady throughout the studied distances. This study concluded that the extent of the cariostatic effect on root dentin was 300 µm for [Ke] and 150 µm for [Fj]. [Dy] and [Su] did not show any cariostatic effect.

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ANEXO 4

Original Paper

Caries Research

Caries Res 2003;37:339–344
DOI: 10.1159/000072165

Received: September 5, 2002
Accepted after revision: March 5, 2003

Caries Progression and Inhibition in Human and Bovine Root Dentine in situ

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Key Words
Bovine dentine · Dentifrice · Fluoride · Human dentine · Root caries

Abstract
Since the use of bovine instead of human dentine to evaluate cariogenic and anticariogenic substances is not well established, this in situ study was conducted. Eleven volunteers wore palatal acrylic devices containing 4 dentine slabs (2 human and 2 bovine). Sucrose solution (20%) was dripped over all slabs 4 times a day, simulating a cariogenic challenge. Dentifrice slurries, fluoridated or not, were dripped over specified dentine slabs 3 times a day to evaluate caries reduction. After 14 days, the biofilm formed on the dentine slabs was collected for microbiological analysis. In dentine, mineral loss (ΔZ) and lesion depth (LD) were determined by cross-sectional microhardness and by polarized light microscopy, respectively. The total streptococci and mutans streptococci counts in the biofilm formed either on human or on bovine slabs, whether treated or not with fluoride dentifrice, were not statistically different. The ΔZ and the LD values of dentine treated with fluoride dentifrice were significantly lower than the values of dentine treated with non-fluoride dentifrice. The differences in the ΔZ and LD values between the human and bovine dentine were not statistically significant. The results suggest that bovine dentine can be used instead of human to evaluate caries development and inhibition.

In situ models have been used to evaluate the cariogenic and anticariogenic properties of fluoride containing dental products [ten Cate, 1994]. This kind of experiment may be considered as an intermediate stage between in vivo and in vitro studies, allowing the control of clinical conditions related to the development of caries [Manning and Edgar, 1992; Zero, 1995; Clasen and Øgaard, 1999]. However, some experimental variables of in situ models need to be standardized [Mellberg, 1992], among them the type of dental substrate. Although there is a consensus that the use of human teeth is more relevant to in situ studies [Zero, 1995], bovine teeth have also been used [Manning and Edgar, 1992; ten Cate and van Duinen, 1995; Zero, 1995; Aires et al., 2002]. The advantage of using bovine instead of human teeth is that they are easier to obtain and to manipulate [Mellberg, 1992]. Moreover, they have a relatively more uniform chemical composi-

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ANEXO 5

COMITÊ DE ÉTICA EM PESQUISA

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "AVALIAÇÃO *IN SITU* DA INDUÇÃO E INIBIÇÃO DE CÁRIE EM DENTINA RADICULAR BOVINA E HUMANA", sob o protocolo nº **017/2002,** do Pesquisador ***ANDERSON TAKEO HARA,*** sob a responsabilidade dos Profs. Drs. **MÔNICA CAMPOS SERRA E JAIME A.PARECIDO CURY,** 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, 03 de abril de 2002

We certify that the research project with title "IN SITU EVALUATION OF CARIES DEVELOPMENT AND INHIBITION IN BOVINE AND HUMAN ROOT DENTIN", protocol nº **017/2002,** by Researcher ***ANDERSON TAKEO HARA,*** responsibility by Prof. Dr. **MÔNICA CAMPOS SERRA** AND **JAIME A.PARECIDO CURY,** is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, April 03 2002

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

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

ANEXO 6

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Detailed Status Information

Manuscript #	04-0284
Current Revision #	0
Submission Date	2004-06-26
Current Stage	Under Consideration
Title	Influence of Fluoride-releasing Restorative Material on Root Dentin Secondary Caries in situ
Running Title	Glass-ionomer cement and secondary caries
Manuscript Type	Research Report
Category	Bio Materials
Manuscript Comment	Number of words in abstract: 150. Number of words in abstract + text: 2500. Number of cited references: 29. Number of tables: 1. Number of figures: 1. Number of appendices: 0.
Corresponding Author	Anderson Hara (State University of Campinas)
Contributing Authors	Anderson Hara, Cecilia Turssi, Masatoshi Ando, Carlos Gonzalez-Cabezas, Domenick Zero, Antonio Rodrigues Jr, Mônica Serra, Jaime Cury
Abstract	This study tested the hypothesis that fluoride-releasing restorations, either aged or un-aged, do not prevent secondary root caries, when fluoride dentifrice is frequently used. Sixteen volunteers wore palatal appliances in 2 phases of 14 days, according to a 2x2 cross-over design. In each phase the appliance was loaded with bovine root dentine slabs restored with either glass-ionomer or resin composite, either aged or un-aged. Specimens were exposed to cariogenic challenge 4x/day and to fluoridated dentifrice 3x/day. The fluoride content in the biofilm (FB) formed on slabs and the mineral loss (DZ) around the restorations were analyzed. No differences were found between restorative materials regarding the FB and the DZ, for both aged ($p=0.792$ and $p=0.645$, respectively) and un- aged ($p=1$ and $p=0.278$, respectively) groups. Under the cariogenic and fluoride dentifrice exposure conditions of this study, the glass-ionomer restoration either aged or un-aged did not provide additional protection against secondary root caries.

ANEXO 7

UNICAMP

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Avaliação in situ do potencial cariostático de materiais restauradores em dentina radicular", sob o protocolo nº **041/2001**, do Pesquisador ***ANDERSON TAKEO HARA***, sob a responsabilidade dos Profs. Drs. **Mônica Campos Serra, Jaime Aparecido Cury** e **Antonio Luiz Rodrigues Jr**, 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, 28 de maio de 2001

We certify that the research project with title "In situ evaluation of the cariostatic effect of restorative materials on root dentin , protocol nº **041/2001,** by Researcher ***ANDERSON TAKEO HARA***, responsibility by Prof. Dr. **Mônica Campos Serra, Jaime Aparecido Cury** and **Antonio Luiz Rodrigues Jr.,** 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 28 2001

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

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