MICHELE BAFFI DINIZ

DETECÇÃO E AVALIAÇÃO DA ATIVIDADE DE LESÕES DE CÁRIE PRIMÁRIA E SECUNDÁRIA EM SUPERFÍCIES OCLUSAIS. ESTUDOS IN VITRO E IN VIVO

ARARAQUARA
2010

unesp UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE ODONTOLOGIA DE ARARAQUARA

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Tese apresentada ao Programa de Pós-Graduação em Ciências Odontológicas, Área de Odontopediatria, da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista "Júlio de Mesquita Filho", para obtenção do título de Doutor em Ciências Odontológicas.

Orientadora: Profª. Drª. Rita de Cássia Loiola Cordeiro

Co-Orientadora: Profª. Drª. Andréa Gonçalves Ferreira Zandoná

Araraquara
2010

Diniz, Michele Baffi

Detecção e avaliação da atividade de lesões de cárie primária e secundária em superfícies oclusais. Estudos in vitro e in vivo / Michele Baffi Diniz.– Araraquara: [s.n.], 2010.

246 f. ; 30 cm.

Tese (Doutorado) – Universidade Estadual Paulista, Faculdade de Odontologia

Orientadora : Profa. Dra. Rita de Cássia Loiola Cordeiro

1. Cárie dentária 2. Diagnóstico 3. Testes de atividade de cárie dentária 4. Fissuras dentárias I. Título

Ficha catalográfica elaborada pela Bibliotecária Marley C. Chiusoli Montagnoli, CRB-8/5646

Serviço Técnico de Biblioteca e Documentação da Faculdade de Odontologia de Araraquara / UNESP

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Araraquara
2010

DADOS CURRICULARES

MICHELE BAFFI DINIZ

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Tua caminhada ainda não terminou....
A realidade te acolhe
dizendo que pela frente
o horizonte da vida necessita
de tuas palavras
e do teu silêncio.

Se amanhã sentires saudades,
lembra-te da fantasia e
sonha com tua próxima vitória.
Vitória que todas as armas do mundo
jamais conseguirão obter,
porque é uma vitória que surge da paz
e não do ressentimento.

É certo que irás encontrar situações
tempestuosas novamente,
mas haverá de ver sempre
o lado bom da chuva que cai
e não a faceta do raio que destrói.

Tu és jovem.
Atender a quem te chama é belo,
lutar por quem te rejeita
é quase chegar a perfeição.
A juventude precisa de sonhos
e se nutrir de lembranças,
assim como o leito dos rios
precisa da água que rola
e o coração necessita de afeto.

Não faças do amanhã
o sinônimo de nunca,
nem o ontem te seja o mesmo
que nunca mais.
Teus passos ficaram.
Olhes para trás...
mas vá em frente
pois há muitos que precisam
que chegues para poderem seguir-te.

Charles Chaplin

DEDICATÓRIA

DEDICO ESTE TRABALHO...

Aos meus queridos pais, Alberto e Marina

Por me presentearem com o maior dos talentos: o dom da vida.

Por me ensinarem desde cedo o significado das palavras determinação e perseverança.

Por me mostrarem a harmonia de nossa profissão: a Odontologia.

Faltam-me palavras para expressar todo o meu amor e gratidão, por tantas demonstrações de carinho, compreensão, renúncias, dedicação, colaboração, incentivo e apoio incondicional que me dispensaram durante toda esta jornada, sempre respeitando minhas decisões. Obrigada por sempre compreenderem a minha necessidade, persistência e até mesmo minha ausência. AMO VOCÊS!!!

Ao meu irmão, Raphael

À minha cunhada, Gigliola

Pela paciência e compreensão durante esses anos.

Pela disposição em me ajudar sempre que precisei.

Obrigada pelo carinho, respeito, amizade e pela constante torcida.

À "quase afilhada", Heloísa

Você é um anjo que entrou na minha vida!

É impossível ficar ao seu lado e não sentir alegria! Te amo pipoquinha!!

Aos meus avós maternos, Waldemar e Joana

Aos meus avós paternos, Rubens (in memoriam) e Lygia

Por incentivarem meus estudos e sempre torcerem por mim em todos os momentos de minha vida.

Pelas demonstrações de bondade, sabedoria e carinho.

Vocês são um exemplo de vida!

ESSA CONQUISTA É DE TODOS

"Sou o que quero ser, porque possuo apenas uma vida e nela só tenho uma chance de fazer o que quero. Tenho felicidade o bastante para fazê-la doce, dificuldades para fazê-la forte, tristeza para fazê-la humana e esperança suficiente para fazê-la feliz.
As pessoas mais felizes não têm as melhores coisas,
elas sabem fazer o melhor das oportunidades que aparecem em seus caminhos."

Clarice Lispector

AGRADECIMENTOS ESPECIAIS

À Deus

Obrigada por me abençoar com uma família marvilhosa. Obrigada pela presença constante, pelas pessoas maravilhosas que colocou em meu caminho, pelos ensinamentos diários e por tudo que me proporciona. Agradeço a oportunidade de realizar meus sonhos, mantendo-me com saúde e dando-me forças nos momentos mais difíceis.

Obrigada Senhor!!

À minha orientadora,

Profª. Drª. Rita de Cássia Loiola Cordeiro

Obrigada pela orientação e apoio em todos os momentos do desenvolvimento deste trabalho. Agradeço as oportunidades, os ensinamentos, a inspiração, as orientações e as cobranças, tão importantes para o meu crescimento e amadurecimento científico. Obrigada por me incentivar a realizar estágio no exterior, pelo apoio nas fases preparatórias e pela motivação e preocupação à distância. Como teria sido difícil sem você... Muito obrigada pela amizade, carinho e dedicação durante esses 8 anos de convivência harmoniosa em Araraquara. Obrigada por despertar meu interesse pela carreira acadêmica, contribuindo para a concretização de um grande sonho. Orgulho-me de tê-la como exemplo de profissional e ser humano!!!

"Feliz aquele que transfere o que sabe e aprende o que ensina"

(Cora Coralina)

À minha co-orientadora,

Profª. Drª. Andréa Gonçalves Ferreira Zandoná

Obrigada pela disponibilidade e confiança durante o meu estágio em Indianápolis, na Universidade de Indiana, EUA. Muito obrigada pelo apoio pessoal dispensado. Agradeço a inestimável contribuição à minha formação profissional e crescimento pessoal, e pela oportunidade de trabalhar sob a sua supervisão, ampliando meus conhecimentos!

Thank you very much!!!

À Profª. Drª. Lourdes Aparecida Martins dos Santos Pinto

Exemplo de professora, educadora e pesquisadora, sempre disposta a ajudar a todos. Obrigada pela convivência, pelo apoio e confiança, pelo crescimento intelectual e profissional e, principalmente, por dividir comigo seus conhecimentos.

Agradeço profundamente!!!

Ao Prof. Dr. Chris Longbottom

Obrigada por compartilhar os conhecimentos científicos e por dedicar parte de seu tempo da Faculdade de Odontologia da Universidade de Dundee, Escócia, em me auxiliar em vários momentos. Agradeço pelo exemplo de pesquisador e pessoa. Obrigada pela oportunidade de trabalharmos juntos, mesmo que à distância, trazendo contribuições valiosas para este trabalho.

Thank you from the deep of my heart!!!

Ao Prof. Dr. Adrian Lussi

Agradeço pelo entusiasmo contagiante pela produção científica, pelo aprendizado, pela disponibilização de equipamentos para a realização deste trabalho e pela convivência agradável durante o meu estágio em seu laboratório. Minha eterna admiração e gratidão!!!

Thank you for all your support!!!

Ao Prof. Dr. Jonas de Almeida Rodrigues

Que sempre compartilhou suas experiências e me apoiou durante minha caminhada na pós graduação. Obrigada pela amizade, convivência e apoio constantes. Obrigada pelos conselhos e incentivos em todos os momentos. Agradeço de todo o meu coração por ter me recebido em sua casa de "portas abertas" em Berna, Suiça, para que pudêssemos trabalhar juntos no início de meu doutorado.

Você foi fundamental para o êxito deste trabalho!!!

Às ***minhas queridas amigas e "irmãs de coração"*** *Cármen Regina Coldebella, Dulce Helena de Oliveira, Mariane Emi Sanabe e Márcia Hiromi Tanaka, por todos os conselhos, pelo apoio constante, pelo carinho e pela amizade sincera. Obrigada pelo convívio maravilhoso que tornou minha vida em Araraquara mais alegre!! ADORO VOCÊS!!!*

"AMIGO, palavra tão fácil de se escrever e pronunciar, mas tão difícil de ter.
AMIGO, é aquele que nos ampara nos momentos difíceis, é aquele que nos crítica nos erros e fraquezas, é aquele que não engana, que não elogia para não explorar.
AMIGO, é aquele que sente a nossa ausência e chora quando choramos.
Um verdadeiro AMIGO é alguém que te conhece tal como és, compreende onde tens estado, acompanha-te em teus lucros e teus fracassos, celebra tuas alegrias, compartilha tua dor e jamais te julga por teus erros".

"'Depois de algum tempo você aprende que verdadeiras amizades continuam a crescer mesmo a longas distâncias, e o que importa não é o que você tem na vida,mas quem você tem na vida."
(W. Shakespeare)

"A maior recompensa para o trabalho do homem não é o que ele ganha com isso,
mas o que ele se torna com isso"

Jhon Ruskin

AGRADECIMENTOS

À ***Universidade Estadual Paulista Júlio de Mesquita – UNESP****, na presença de seu Magnífico Reitor Prof. Dr. Herman Jacobus Cornelis Voorwald e Vice-Reitor, Prof. Dr. Julio Cezar Durigan.*

À ***Faculdade de Odontologia de Araraquara - FOAr*** *da Universidade Estadual Paulista Júlio de Mesquita Filho - UNESP, representados pelo digníssimo Diretor Prof. Dr. José Cláudio Martins Segalla e pela Vice-Diretora, Profª. Drª. Andréia Affonso Barreto Montandon.*

Ao ***Departamento de Clínica Infantil*** *da Faculdade de Odontologia de Araraquara - UNESP, representado pelo Chefe de Departamento Prof. Dr. Luis Gonzaga Gandini Júnior e pela Vice-Chefe Profª. Drª. Angela Cristina Cilense Zuanon.*

Aos ***docentes da Disciplina de Odontopediatria*** *da Faculdade de Odontologia de Araraquara - UNESP, Profª. Drª. Angela Cristina Cilense Zuanon, Prof. Dr. Cyneu Aguiar Pansani, Profª. Drª. Elisa Maria Aparecida Giro, Prof. Dr. Fábio Cesar Braga de Abreu e Lima, Profª. Drª. Josimeri Hebling, Profª. Drª. Lourdes Aparecida Martins dos Santos Pinto e Profª. Drª. Rita de Cássia Loiola Cordeiro, pela convivência diária e contribuição à minha formação pessoal e profissional. Obrigada por me mostrarem o caminho maravilhoso da Odontopediatria!!*

"A tarefa essencial do professor é despertar a alegria de trabalhar e de conhecer."

(Albert Eisntein)

Aos ***docentes da Disciplina de Ortodontia*** *da Faculdade de Odontologia de Araraquara - UNESP, Prof. Dr. Ary dos Santos Pinto, Prof. Dr. Dirceu Barnabé Raveli, Prof. Dr. João Roberto Gonçalves, Profª. Drª. Lídia Parsekian Martins, Prof. Dr. Luiz Gonzaga Gandini Júnior e Prof. Dr. Maurício Tatsuei Sakima, pela convivência agradável.*

Ao ***Programa de Pós-Graduação em Ciências Odontológicas*** *da Faculdade de Odontologia de Araraquara - UNESP, representado pela Coordenadora Profª. Drª. Josimeri Hebling e pelo Vice-Coordenador Prof. Dr. Edson Alves de Campos, pela oportunidade de ser aluna deste programa.*

*Aos **professores do Programa de Pós-Graduação** em Ciências Odontológicas da Faculdade de Odontologia de Araraquara - UNESP, pela atenção e disponibilidade.*

*Aos **funcionários do setor de Pós-Graduação** da Faculdade de Odontologia de Araraquara - UNESP, Flávia Sousa de Jesus, José Alexandre Garcia, Mara Cândida Munhoz do Amaral e Rosângela Aparecida Silva dos Santos, pela constante ajuda, atenção e organização em todos os momentos.*

*Aos **funcionários do Departamento de Clínica Infantil** da Faculdade de Odontologia de Araraquara - UNESP, Antonio Parciaseppe Cabrini (Totó), Cristina Ferreira Affonso, Dulce Helena de Oliveira, Marcia Helena Caravalho Held, Odete Amaral (Dona Odete), Pedro César Alves (Pedrinho), Sônia Maria Tircailo (Soninha) e Tânia Aparecida Moreira dos Santos, pelo convívio agradável e por sempre nos auxiliarem.*

*Aos **funcionários da Biblioteca** da Faculdade de Odontologia de Araraquara - UNESP, Adriano Ferreira Luiz, Ceres Maria Carvalho Galvão de Freitas, Eliane Cristina Marques de Mendonça Spera, Eliane Maria Sanches Scarso, Maria Aparecida Capela Carvalho, Maria Inês Carlos, Silvia Helena Acquarone Lavras e Odete Aparecida Camilo, por estarem sempre dispostos a nos ajudar. Em especial à Marley Cristina Chiusoli Montagnoli, pelo auxílio na formatação desse trabalho e pela elaboração da ficha catalográfica.*

*Às **colegas de turma do curso de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontopediatria, Nível Doutorado** da Faculdade de Odontologia de Araraquara - UNESP, Cármen Regina Coldebella, Juliana Oliveira Gondim, Nancy Tomoko Sacono e Simone Di Salvo Mastrantonio, pelo companheirismo, apoio e convivência em Araraquara durante nossa vida acadêmica.*

Aos ***colegas de outras turmas do curso de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontopediatria, Nível Mestrado e Doutorado*** *da Faculdade de Odontologia de Araraquara - UNESP, Amanda Fontana, Ana Luísa Botta Martins de Oliveira, Ana Paula Silveira Turrioni, Andreza Maria Fábio Aranha, Beatriz Ferraz dos Santos, Camila Fávero de Oliveira, Camila Maria Bullio Fragelli, Célia Lanza, Cristiane Duque, Cristiane Maria da Costa Silva, Cristiane Motisuki, Débora Lopes Salles Scheffel, Elcilaine Rizatto Azevedo, Érika Botelho Josgrilberg, Fabiano Jeremias, Fábio Luiz Scannavino, Fernanda Campos Rosetti Lessa, Hérica Adad Ricci, Hermes Pretel, Indri Nogueira, Jonas de Almeida Rodrigues, Juliana Feltrin de Souza, Junia Carolina Linhares Ferrari dos Santos, Leticia Vargas Freire Martins Lemos, Lícia Bezerra Cavalcante, Luciana Bianchi, Luciana Monti Lima, Marcela Martini Tagliani, Márcia Hiromi Tanaka, Marco Aurélio Benini Paschoal, Margareth da Mata, Mariane Emi Sanabe, Marília Ferreira Correia, Murilo de Sousa Guimarães, Natália Apolinário de Lima, Thalita Boldieri, pela atenção e agradável convivência no Departamento.*

Aos ***colegas do curso de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Ortodontia e Dentística Restauradora, Nível Mestrado e Doutorado*** *da Faculdade de Odontologia de Araraquara - UNESP, pela convivência nas Disciplinas e nas atividades científicas.*

Às ***estagiárias da Disciplina de Odontopediatria*** *da Faculdade de Odontologia de Araraquara - UNESP, do ano de 2007 e 2008, Adriana Regina Colombo Pauleto, Ana Paula Kovacs, Camila Andrieli Cury, Carolina Ormon Giacon, Daniella de Giácomo Caetano, Fabiana Bueno de Godoy Campos, Fernanda C. de Carvalho, Gabriela Santos Reis Adrega de Moura, Gisele Ortolan Bueno, Keli Regina Victorino, Keren Cristina Fagundes Jordão Basso, Larissa Stangari, Lígia Nunes de Morais Ribeiro, Mariana Fiochi Pena, Marilis D. Conti, Natália Apolinário de Lima e Paloma Gagliardi Minotti, pela amizade que se estendeu além do estágio e pela convivência agradável nos dias de clínica.*

Ao ***Departamento de Diagnóstico e Cirurgia*** *da Faculdade de Odontologia de Araraquara – UNESP,* ***em especial aos professores da Disciplina de Cirurgia e Traumatologia Buco-Maxilo-Facial****, Prof. Dr. Eduardo Hochuli Vieira, Prof. Dr. José Scarso Filho, Prof. Dr. Mário Francisco Real Gabrielli, Profª. Drª. Marisa Aparecida Cabrini Gabrielli, Prof. Dr. Roberto Henrique Barbeiro e Prof. Dr. Valfrido Antonio Pereira Filho,* ***aos residentes*** *Henrique do Couto de Oliveira, João Octávio Pompeu Hyppolito, Lucas Martins de Castro e Silva, Nicolau Conte Neto, Waloner Ricardo Souza de Caravalho e Willian Morais de Melo,* ***aos estagiários*** *Fernanda Daura Jorge Brasil Boos, Marcos Vinicius Mendes Dantas e Vitor Hugo Oliveira Amorim,* ***aos técnicos em radiologia*** *Edneide Alves Ferreira e Marcos Dimas Olivi e* ***às funcionárias*** *Isabela Cristine Manzolli e Silvana Aparecida Rodrigues Camargo Pelaes, por me "acolherem" prontamente e me ajudarem em todos os momentos para a realização de parte deste trabalho.*

Aos ***colaboradores deste trabalho,*** *Plínio Sciasci e Thalita Boldieri, pela convivência harmoniosa, amizade e dedicação. Obrigada por todo apoio e por sempre estarem ao meu lado!*

Ao ***grupo de pesquisa do CNPq "Cárie dentária: métodos e critérios de avaliação",*** *coordenado pelas Profª. Drª. Lourdes Aparecida Martins dos Santos Pinto e Profª. Drª. Rita de Cássia Loiola Cordeiro, com a participação da Profª. Drª. Luciana Monti Lima e dos alunos da graduação Ana Bárbara de Araújo Loiola, Chaiene Evelin Zago, Diego Girotto Bussaneli, Fernando José Lopes de Campos Carvalho, João Flávio Nishihara Pinto Rodrigues, Julia Olien Sanches, Livia Etchebehere Loiola, Mayara Liboni Gandia, Plínio Sciasci, e Thalita Boldieri, pela ótima convivência, pelo prazero ambiente de trabalho e pela dedicação ao grupo.*

Foi um imenso prazer trabalhar com vocês!!

Aos ***amigos e funcionários da Faculdade de Odontologia da Universidade de Berna, Suiça****, Brigitte Megert, Isabel Hug, Klaus Neuhaus, Simon Flury, Zeinab Cheaib e Wale Buergin, por terem me recebido com tanto carinho em Berna, pela atenção e disponibilidade durante a execução de projetos na Suiça.*

Thank you for your kindness and support!!

Aos ***amigos da Suiça,*** *Cristiano Faria Maciel, Jürg Michael Hirschi e Mariana Cristina de Almeida Vieira, pela amizade sincera e por terem me ajudado no dia a dia com as dificuldades encontradas no exterior. Agradeço também pela agradável companhia em nossas viagens.*

Vou guardar para sempre essas lembranças...

À ***amiga Andreza Maria Fábio Aranha****, que desde meu estágio em Araraquara se mostrou uma pessoa íntegra, amiga e dedicada. Muito obrigada pelo apoio, força, dicas, conselhos e carinho durante minha experiência no exterior. Jamais me esquecerei. Te adoro!!*

À ***querida amiga e companheira Cristiane Tomaz Rocha****, que desde o princípio abriu o coração para uma amizade verdadeira. Obrigada pelo carinho e por sempre me incentivar a lutar pelos meus ideais. Obrigada por estar sempre ao meu lado nos momentos de alegria e dificuldades durante nosso estágio no exterior.*

"Conhecer alguém aqui e ali que pensa e sente como nós, e que embora distante, está perto em espírito, eis o que faz da Terra um jardim habitado"

(Goethe)

Aos ***professores do Oral Health Research Institute*** *da Faculdade de Odontologia da Universidade de Indiana, Indianápolis, IN, EUA, Profª. Ana Gossweiler, Prof. Dr. Anderson T. Hara, Profª. Drª. Andréa Ferreira Zandoná, Prof. Dr. Armando Soto Rojas, Prof. Dr. Domenick Zero, Profª. Drª. Esmeralda Angeles Martínes Mier, Prof. Dr. Frank Lippert, Prof. Dr. Gerardo Maupomé, Prof. Dr. Masatoshi Ando, Prof. Dr. Richard Gregory e Prof. Dr. Richard Jackson, pela atenção, conhecimentos transmitidos e auxílio durante a realização desta pesquisa. Thank you for accepting me in your Department and all support to reach my goals!!!*

It was an honor to work with your research group!!!

Aos ***funcionários do Oral Health Research Institute*** *da Faculdade de Odontologia da Universidade de Indiana, Indianápolis, IN, EUA, em especial do* ***Specimen Processing Laboratory****, Brenda Conder, Chris Buckley, Joseph Joseph, Scott Yao, Wenbo Yao e Yuan Chen, do* ***Demin/Remin Fluoride Laboratory for contract testing****, Grishma Patel, Jennifer Eder, LaTia Mans e Rolando Torres, do* ***Fluoride Research Laboratory,*** *Chris Buckley e Prithvi Chandrappa, do* ***Hardness Evaluation, Pellicle Cleaning and TMR Laboratory,*** *Clif Duhn, Glynis Henry, Kathy Wagner, Nyinyi Tin, Marharyta Isiutsina e Rose Hinesley, do* ***Abrasion/Pellicle Cleaning Laboratory,*** *Kelly Perkins, Ro Ding, Tara Edmundson e Terry Ball, do* ***Erosion Research Laboratory,*** *Adam Kelly e May Hliang, da* ***Central Services and Infection Control,*** *Anneta Hall, Mariamma Joseph, Mike Johnston, Sharon Gwinn, Tasha Terhune, Van Bualteng e Weiping Zhang, do* ***Clinical Research Facility****, Charlene Ellis, Druscilla Kriech, Jennifer Tran, Jodie Jarrett, Judy Burch, Liliya Lukantsova, Melissa Mau, Sue Kelly e Susan Boggs, do* ***OHRI Administrative Staff,*** *Beverly Spriggs, Latonya Kirkland, Marilyn Richards e Vicky Jennings, por terem me recebido com tanto carinho em Indianápolis, pela atenção e disponibilidade durante a execução de meus experimentos, pelo auxílio na realização das análises laboratoriais, pela atenção e amizade, tornando o meu dia a dia mais agradável.*

Thank you for giving me all the professional support I needed!!!

Ao ***Prof. Dr. Carlos González-Cabezas*** *da Faculdade de Odontologia da Universidade de Michigan, Ann Arbor, MI, EUA, pelos ensinamentos fundamentais e pela disposição em me ajudar em alguns experimentos.*

Thank you very much for sharing with me your knowledge concerning secondary caries research!!!

Aos ***colegas de trabalho do Oral Health Research Institute*** *da Faculdade de Odontologia da Universidade de Indiana, Indianápolis, IN, EUA, Julie Breckenridge, Olga Isiutsina e Tadziu Kula III, pela convivência e pelos bons momentos compartilhados na sala de computadores do OHRI.*

Thank you for the attention!!!

*Ao **Amir Haider,** analista de pesquisa do Microbial Caries Laboratory da Faculdade de Odontologia da Universidade de Indiana, Indianápolis, IN, EUA, pelo carisma e disposição em me ajudar durante a análise dos espécimes utilizando o Confocal Laser Scanning Microscopy (CLSM) no Departamento de Nefrologia da Faculdade de Medicina da Universidade de Indiana, Indianápolis, IN, EUA.*

I really appreciate your help and availability!!

*À **Brigitte Marie Dan,** técnica do Oral Health Research Institute da Faculdade de Odontologia da Universidade de Indiana, Indianápolis, IN, EUA, que além de amiga e companheira de trabalho, teve participação importante durante a análise com o aparelho Quantitative Light-induced Fluorescence (QLF).*

Thank you for your support, my friend!!

*Às **queridas amigas de Indianápolis** Cristiane Sá Roriz Fonteles e Silvia Franklin, pela amizade sincera, pelo apoio incondicional e por terem me acolhido tão carinhosamente, tornando minha estada em Indianápolis, IN, EUA ainda mais prazerosa e aconchegante.*

Minha eterna gratidão!!

*Às **eternas amigas de Indianápolis** Oriana Reis Capin (Ori) e Marília Mattos Sly (Lila), pela amizade, carinho e ensinamentos. Estímulos constante para minha pesquisa e para enfrentar os desafios no exterior, possuidoras de um carisma e uma simpatia inigualáveis!!*

*Às **colegas brasileiras dos EUA** Ana Maria Spor, Carolina Steiner de Oliveira, Sibele Pereira de Oliveira, Taís Scaramucci e Thyciana Ribeiro, pelo apoio, companheirismo e força durante minha estada em Indianápolis, IN, EUA.*

Aos ***professores, alunos da graduação e da pós-graduação da Indiana University School of Dentistry, Indianapólis, IN, EUA,*** *por participarem como examinadores voluntários de um projeto de pesquisa, dedicando parte de seu tempo à realização de meus trabalhos.*

I had a great time working with you!!!

Ao ***George Joseph Eckert*** *da Faculdade de Medicina da Universidade de Indiana, Indianápolis, IN, EUA, pela disponibilidade e realização cuidadosa da análise estatística de parte deste trabalho.*

Thank you for your attention!!

Ao ***Prof. Dr. Nigel Pitts,*** *da Faculdade de Odontologia da Universidade de Dundee, Escócia, pela contribuição científica para a realização de parte deste trabalho.*

Thank you so much!!!

Às ***pesquisadoras da LUX DS****, Edinburgh, Reino Unido, Emma Perfect e Annie Marie Haughey pela atenção dispensada e pelos ensinamentos sobre a nova tecnologia baseada em luminescência para avaliação da atividade de lesões de cárie.*

Thank you for your support!!!

À ***Profª. Drª. Josimeri Hebling****, pelas valorosas sugestões durante o processo de qualificação e pelo exemplo de pesquisadora e pessoa!!*

Ao ***Prof. Dr. Fausto Medeiros Mendes****. Agradeço pela atenção, disponibilidade e por ter nos auxiliado sempre quando precisamos. Agradeço pelo empréstimo da ponta do DIAGNOdent para realização de parte deste trabalho. Obrigada pelo carinho e por sempre se mostrar solícito!!*

Tenho uma enorme admiração por você!!

*À **Profª. Drª. Denise Fonseca Côrtes**, pelo carinho com que sempre nos recebeu e por confiar em nossos trabalhos. Muito obrigada!*

*Ao **meu querido amigo Cristiano Faria Maciel**, que sabe como ninguém realizar trabalhos ilustrativos e compreensíveis, dando mais harmonia e riqueza ao trabalho científico. Obrigada por trabalhar com tanto carinho e disposição na confecção da capa e contra capas deste trabalho!*

*À **colega de profissão Sônia Burgati,** pela dedicação à Odontopediatria e pelo exemplo de profissional!! Obrigada pelo apoio e carinho!!*

*Às **amigas** Andréia Bolzan de Paula, Luciana Monti Lima, Mayara Aguilar Dias de Brito, Mirela de Barros Tamarozzi e Ticiana Sidorenko de Oliveira Capote, pela amizade sincera e por todo apoio, carinho e alegria. Adoro vocês!!*

"A amizade só podia ter lugar através do desenvolvimento do respeito mútuo e dentro de um espírito de sinceridade."

(Dalai Lama)

*Aos **amigos** Franklin Forte, Marco Aurélio Rodrigues, Rafael G. Azeredo e Pedro Luis Fagá Celli, pelo convívio agradável e apoio em todos esses anos. Obrigada pelo apoio durante meu estágio no exterior!*

*À **CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior**, pela concessão de bolsa de estudo que possibilitou a cobertura financeira desta pesquisa durante o curso de Doutorado no Brasil e pela concessão de bolsa de estudo com Estágio de Doutorado no Exterior – PDEE (Processo BEX nº 4053/08-7), que possibilitou a realização de parte deste trabalho no Department of Preventive and Community Dentistry – Oral Health Research Institute, Indiana University School of Dentistry, Indianapolis, IN, USA.*

*À **FAPESP** – Fundação de Amparo à Pesquisa do Estado de São Paulo, pela concessão de Auxílio Pesquisa Regular (Processo nº 2009/00218-0) para realização de parte desse trabalho no Brasil*

*À **Universidade de Berna, Suiça e à Universidade de Indiana, EUA,** por terem me recebido prontamente para a realização de estágio no exterior.*

*Aos **pacientes do Departamento de Clínica Infantil e do Departamento de Diagnóstico e Cirurgia** da Faculdade de Odontologia de Araraquara - UNESP, pela colaboração e participação essenciais para a execução desta pesquisa.*

*Aos **pacientes** que doaram seus dentes para a realização deste trabalho.*

*Aos **meus familiares, amigos e a todos** que, direta ou indiretamente, colaboraram para a execução desta pesquisa.*

MUITO OBRIGADA!!

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RESUMO

Diniz MB. Detecção e avaliação da atividade de lesões de cárie primária e secundária em superfícies oclusais. Estudos in vitro e in vivo [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2010.

Resumo

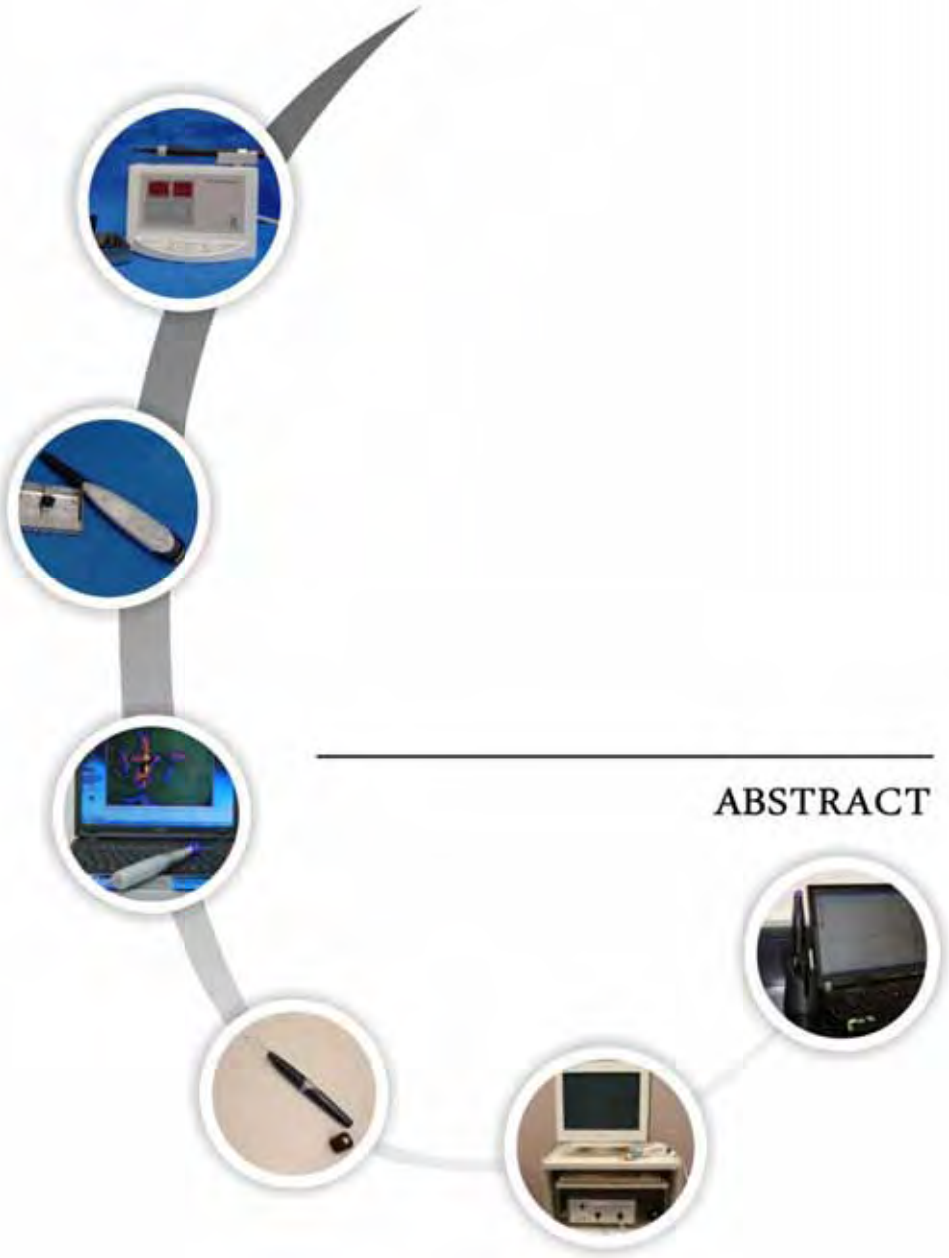
O objetivo do presente estudo foi avaliar a efetividade de métodos empregados na detecção e na avaliação da atividade de lesões de cárie primárias e secundárias em superfícies oclusais de dentes decíduos e permanentes. Para tanto, foram realizadas quatro pesquisas. (1) O objetivo desta pesquisa foi avaliar in vitro a influência de métodos de profilaxia profissional nas medidas de fluorescência e no desempenho de três aparelhos baseados na captação da fluorescência induzida por luz na detecção de lesões de cárie oclusal em dentes permanentes. Os exames foram realizados em 110 dentes permanentes por dois examinadores no início, após a profilaxia profissional e lavagem e após uma segunda lavagem. Foram utilizados os aparelhos DD (DIAGNOdent 2095, KaVo, Alemanha), DD*pen* (DIAGNOdent 2190, KaVo, Alemanha) e VP (VistaProof, Dürr Dental, Alemanha). Os dentes foram aleatoriamente distribuídos de acordo com o método de profilaxia profissional empregado: jato de bicarbonato de sódio ou pasta profilática. Pode-se concluir que a escolha do método de profilaxia professional pode influenciar significativamente as medidas de fluorescência e o desempenho de métodos baseados na captação da fluorescência

induzida por luz na detecção de lesões de cárie oclusal. (2) O objetivo deste estudo in vivo foi determinar os pontos de corte ideais para o DD, o DD*pen* e a VP, e avaliar a validade clínica de métodos convencionais e de métodos baseados na captação da fluorescência induzida por luz na detecção de lesões de cárie oclusal em dentes permanentes. Foram selecionados 105 dentes permanentes posteriores indicados para extração em 88 pacientes adultos. Um examinador experiente avaliou os dentes usando métodos baseados na captação da fluorescência induzida por luz (DD, DD*pen* e VP), ICDAS e exame radiográfico (Rx). Pode ser concluído que o ICDAS, o DD e o DD*pen* apresentaram boa validade em detectar lesões de cárie oclusal in vivo. Entretanto, o Rx e a VP foram pouco eficientes em detectar, respectivamente, todas os tipos de lesões e lesões em dentina. Além disso, os pontos de corte dos métodos baseados na captação da fluorescência induzida por luz não devem ser considerados pelos dentistas como limiares exatos na decisão de tratamento. (3) Os objetivos deste estudo foram comparar in vivo dois critérios clínicos para avaliação de atividade de lesões de cárie, comparar esses resultados in vivo com os resultados in vitro obtidos pelo novo método baseado em luminescência e comparar ambos critérios com esse método para determinar o status da atividade de lesões de cárie reexaminadas após 2 meses de acompanhamento clínico. Os dois critérios clínicos foram comparados em 88 molares decíduos de 58 crianças com

idade entre 9 e 12 anos. Uma parte da amostra (n=10) foi reexaminada após 2 meses. Após a esfoliação ou extração, os dentes foram analisados por um novo método baseado em luminescência (Carivis, LUX DS, Reino Unido). Em conclusão, os critérios clínicos apresentaram uma alta correlação quando empregados in vivo. Sugere-se que o método de luminescência apresente capacidade em avaliar a atividade de lesões de cárie in vitro. (4) Neste estudo in vitro foi avaliado o desempenho do critério visual ICDAS e de novas tecnologias na detecção de lesões de cárie natural ao redor de restaurações de amálgama e de resina composta em dentes permanentes e determinada a relação entre a presença da lesão de cárie secundária e a presença/ausência de diferentes tamanhos de defeitos marginais. Foram selecionados cento e oitenta dentes com restaurações de amálgama (n=90) e de resina composta (n=90). Dois examinadores analisaram os dentes utilizando o critério visual ICDAS, o aparelho de fluorescência a laser (DD), o aparelho baseado em LED (Midwest Caries I.D., DENTSPLY Professional, EUA), o aparelho QLF (QLF-clin, Inspektor Research Systems BV, Holanda) e um protótipo baseado na tecnologia da imagem da fluorescência do esmalte (PCDS, Therametrics Technologies, EUA). O tamanho do defeito marginal foi analisado por meio de uma sonda exploradora. O padrão outro foi determinado pela microscopia confocal de varredura a laser (CLSM). Em conclusão, o ICDAS e o DD apresentaram bom desempenho na detecção de

lesões de cárie ao redor de restaurações de resina composta, e o QLF e o PCDS na detecção de lesões de cárie ao redor de restaurações de amálgama e de resina composta. O tamanho do defeito marginal foi irrelevante na determinação da presença de lesões de cárie ao redor de restaurações. Por meio desta pesquisa, observou-se que os métodos descritos acima para detecção e avaliação da atividade de lesões de cárie parecem contribuir de forma positiva no processo de diagnóstico. No entanto, devem ser considerados como auxiliares aos métodos convencionais nesse processo.

Palavras chave: Cárie dentária, Diagnóstico, Testes de atividade de cárie dentária, Fissuras dentárias



ABSTRACT

Diniz MB. Detection and activity assessment of primary and secondary caries on occlusal surfaces. In vitro and in vivo studies. [Tese de Doutorado]. Araraquara: Faculadde de Odontologia da UNESP; 2010.

Abstract

The aim of this study was to evaluate the effectiveness of methods for caries detection and caries activity assessment on occlusal surfaces of primary and secondary caries in deciduous and permanent teeth. For this reason, four studies were carried out: (1) The aim of this study was to evaluate the effects of professional prophylaxis procedures on fluorescence measurements and on three fluorescence-based devices in detecting occlusal caries in permanent teeth. All assessments were performed in 110 permanent teeth by two examiners at baseline, after professional prophylaxis and rinsing, and after a second rinsing. The devices LF (DIAGNOdent 2095, KaVo, Germany), LFpen (DIAGNOdent 2190, KaVo, Germany) and FC (VistaProof, Dürr Dental, Germany) were used. The teeth were randomly divided according to the professional prophylaxis used: sodium bicarbonate jet or prophylaxis paste. It can be concluded that the choice of professional prophylaxis method can significantly influence the fluorescence measurements and the performance of fluorescence-based methods for detection of occlusal caries. (2) This in vivo study proposed clinical cut-off limits for three fluorescence-

based methods and evaluated the validity of conventional and fluorescence-based methods for occlusal caries detection in permanent teeth. A total of 105 posterior permanent teeth with an indication for extraction were selected in 88 adult patients. One experienced examiner assessed the teeth using fluorescence-based methods (LF, LFpen and FC), ICDAS and bitewing radiographs (BW). It can be concluded that ICDAS, LF and LFpen demonstrated good validity in detecting occlusal caries in vivo. However, BW and FC showed the lowest validity to detect all lesions and dentin lesions, respectively. Besides, the fluorescence-based methods cut-off limits should not be considered as exact thresholds for dentists' treatment decision. (3) The aims of this study were to compare two clinical criteria for caries activity assessment in primary teeth in vivo, to compare these in vivo results with the in vitro results obtained by a novel luminescence assay, and to compare both criteria with the luminescence assay to determine the activity status on lesions subject to a 2-month in vivo follow-up. The two in vivo criteria were compared in 88 primary molars of 58 children aged 9-12 years. A subsample (n=10) was re-examined after 2 months. After exfoliation or extraction, the teeth were evaluated by a novel luminescence assay (Carivis, LUX DS, United Kingdom). In conclusion, the clinical criteria exhibited a high correlation when applied in vivo. It could be suggested that the luminescence assay presents ability to assess caries activity status in vitro. (4) This in vitro

study evaluated the performance of the ICDAS visual criteria and new technologies for detecting natural caries around amalgam and composite resin restorations in permanent teeth, and the relationship between the presence of caries around restorations and the presence/absence of different gap sizes. One hundred and eighty teeth with amalgam (n=90) and composite resin (n=90) restorations were selected. Two examiners analyzed the teeth using the ICDAS visual criterion, laser fluorescence (LF), LED device (MID) (Midwest Caries I.D., DENTSPLY Professional, USA), quantitative light-induced fluorescence system (QLF) (QLF-clin, Inspektor Research Systems BV, The Netherlands) and a prototype system based on the Fluorescence Enamel Imaging (PCDS) (Professional Caries Detection System, Therametrics Technologies, USA). Additionally, gap size was evaluated using an explorer probe. The gold-standard was determined by means of confocal laser scanning microscopy (CLSM). In conclusion, ICDAS and LF presented good ability to detect caries around composite resin restorations, and QLF and PCDS to detect caries around amalgam and composite resin restorations. Gap size was found to be irrelevant to determine the presence of caries around restorations. From this study, it could be observed that the methods described above for detection and activity assessment appear to contribute positively in the diagnosis process. However, they should be considered as adjuncts to conventional methods in this process.

Keywords: Dental Caries, Diagnosis, Dental Caries Activity Tests, Dental Fissures

INTRODUÇÃO

Introdução

A lesão de cárie é o sinal clínico da doença cárie dentária, podendo apresentar-se como mudanças iniciais no conteúdo mineral, situadas em nível ultraestrutural, até a destruição total da estrutura dentária (Fejerskov [23], 1997). As lesões de cárie podem ser classificadas como primárias ou secundárias para diferenciar as lesões presentes em superfícies não restauradas daquelas que se desenvolvem adjacentes às restaurações dentárias (Kidd, Fejerskov[36], 2004). Além disso, uma lesão de cárie ativa é caracterizada por apresentar predominância de perda de minerais, enquanto que uma lesão inativa apresenta maior predominância de períodos de deposição de minerais (Nyvad, Fejerskov[55], 1997).

As superfícies oclusais têm sido demonstradas como os locais mais susceptíveis para o desenvolvimento da lesão de cárie primária devido às características morfológicas do sistema de fóssulas e fissuras que favorece o acúmulo de placa bacteriana (Beltrán-Aguilar et al.[9], 2005). Embora as lesões de cárie secundária sejam mais comumente encontradas nas margens gengivais de restaurações Classe II e V, lesões na superfície oclusal também podem ser detectadas, especialmente ao redor de restaurações de resina composta (Mjör[53], 1985; Mjör [52], 2005).

A ampla utilização e disponibilidade dos fluoretos têm contribuído efetivamente para as mudanças no desenvolvimento e na progressão da lesão de cárie nas superfícies oclusais (Angmar-Månsson et al.[4], 1998; Cury et al.[16], 2004), uma vez que o flúor remineraliza a camada mais superficial do esmalte, enquanto a lesão progride na sua subsuperfície, caracterizando as lesões de cárie oculta. Assim, as lesões não cavitadas ou em estágios iniciais de desenvolvimento tornaram-se mais prevalentes que as lesões cariosas cavitadas (Ekstrand et al.[21], 1998; Heinrich-Weltzien et al.[28], 2003).

Conseqüentemente, a progressão mais lenta da lesão de cárie favorece sua detecção precocemente, possibilitando o correto manejo do paciente por meio de medidas preventivas e evitando-se assim, a prática da filosofia de intervenção restauradora (Pretty, Maupomé [59], 2004). No entanto, o diagnóstico e o estabelecimento do plano de tratamento ainda constituem um grande desafio aos cirurgiões dentistas.

É importante enfatizar que o diagnóstico da doença cárie é um procedimento que implica não somente na detecção e determinação da extensão das lesões, mas também, na avaliação da atividade destas lesões. O conhecimento da natureza dinâmica do desenvolvimento da lesão de cárie é essencial para avaliar seu status, permitindo que uma lesão ativa se torne inativa (Nyvad, Fejerskov[55], 1997).

O método de detecção da lesão de cárie ideal deve ser acurado, de simples aplicação e indicado para todas as superfícies dentárias. Entretanto, esse método não deve somente auxiliar na detecção da lesão de cárie primária, mas também no de cárie secundária, que é o principal motivo de substituições de restaurações dentárias (Kidd et al.[35], 1992). A detecção de cárie secundária em estágios iniciais é extremamente complicada, uma vez que colorações próximas às restaurações ou margens pigmentadas ou defeituosas não indicam necessariamente a presença de lesão (Kidd, Beighton[34], 1996).

Tradicionalmente, o exame visual e o exame radiográfico constituem os métodos mais empregados para a detecção de lesões de cárie primária e secundária na clínica odontológica. Embora o exame tátil não seja um método acurado, ainda constitui o método mais tradicional associado ao exame visual para a detecção de cárie secundária (Ando et al.[2], 2004). Contudo, o exame visual baseia-se em critérios subjetivos como cor, translucidez e dureza da estrutura dentária, apresentando alta especificidade e baixa sensibilidade (Lussi[50], 1991; Pretty, Maupomé[59], 2004). Por esse motivo, alguns critérios têm sido propostos com o objetivo de reduzir sua subjetividade, aumentar a sensibilidade, possibilitar o monitoramento de lesões em estágios iniciais, além de avaliar a atividade da lesão (Ekstrand et al.[22], 1997; Nyvad et al.[56], 1999).

O ICDAS (*International Caries Detection and Assessment System*) foi desenvolvido recentemente por um grupo de pesquisadores com o objetivo de estabelecer um critério visual internacional para codificar os sinais clínicos das lesões de cárie, permitindo a padronização dos dados coletados e sua comparação entre os estudos laboratoriais, clínicos, levantamentos epidemiológicos e monitoramento de pacientes durante a prática clínica pública e privada. O princípio fundamental desse critério consiste em um exame visual da superfície dentária limpa e seca para inicialmente avaliar a condição do elemento dentário (hígido, restaurado, selado, com coroa ou ausente), e posteriormente, avaliar a presença ou ausência de lesões de cárie nas superfícies, que são classificadas por meio de sete códigos, variando desde hígido à extensa cavitação com envolvimento de dentina (Zandoná, Zero[69], 2006; Ekstrand et al.[20], 2007; Ismail et al.[31], 2007).

Estudos têm mostrado que o ICDAS apresenta boa reprodutibilidade e acurácia para detecção de lesões de cárie oclusal em dentes permanentes (Ekstrand et al.[20], 2007; Ismail et al.[31], 2007; Jablonski-Momeni et al.[32], 2008; Rodrigues et al.[61], 2008; Diniz et al.[19], 2009) e em dentes decíduos (Shoiab et al.[64], 2009; Braga et al.[12], 2009; Neuhaus et al.[54], 2010), e boa reprodutibilidade em estudos epidemiológicos (Finlayson et al.[24], 2007; Sohn et al.[65], 2007; Kühnisch et al.[39], 2008; Braga et al.[14], 2009). Embora esse critério esteja indicado para avaliar dentes com restaurações ou selantes,

nenhum trabalho avaliou sua efetividade na detecção de lesões de cárie secundária. Além disso, estudos clínicos são necessários para validar o critério na detecção de lesões de cárie primária.

Como o critério ICDAS não pode ser empregado diretamente para avaliar a atividade de lesões de cárie, um novo critério foi estabelecido para ser utilizado em associação ao ICDAS (Ekstrand et al.[20], 2007). O LAA (*Lesion Activity Assessment*) baseia-se na análise de vários parâmetros, como a classificação da lesão de cárie pelo critério ICDAS, a localização ou não da lesão em áreas de estagnação de placa e a avaliação da textura da superfície pela sensação táctil de uma sonda de ponta romba. Cada um desses parâmetros é convertido em escores específicos, e a soma deles indicará a atividade da lesão de cárie. A associação de ambos critérios permite detectar a lesão, estimar sua severidade e avaliar sua atividade, que são fatores fundamentais para o correto manejo do paciente (Braga et al.[12], 2009). Ekstrand et al.[20] (2007) observaram que o LAA apresentou boa acurácia na determinação da atividade de lesões de cárie in vivo. Em um estudo laboratorial observou-se boa correlação para avaliação da atividade de cárie em superfícies oclusais de dentes decíduos (Braga et al.[12], 2009).

Recentemente, outro método foi desenvolvido para avaliar a atividade de lesões de cárie. O Carivis™ (LUX DS, Lux Innovate Ltd, Edinburgh, Reino Unido) é um método baseado em luminescência que utiliza um marcador de

íons cálcio denominado Glowdent™ (LUX DS, Lux Innovate Ltd, Edinburgh, Reino Unido), que tem a capacidade de capturar os minerais liberados durante o processo de desmineralização dentária e emitir um sinal luminoso. Este sinal é capturado por um dispositivo intraoral e uma imagem é produzida (Longbottom et al.[42], 2008). Como a quantidade de luz emitida é proporcional à quantidade de minerais presentes, a imagem resultante oferece um "mapa da desmineralização dentária", auxiliando o profissional na determinação da localização, tamanho e extensão da perda mineral em lesões de cárie ativas, permitindo o monitoramento do paciente e o estabelecimento do plano de tratamento mais adequado. Alguns estudos laboratoriais têm mostrado um bom desempenho do marcador luminescente de íons cálcio como um possível avaliador da atividade de lesões de cárie em dentes decíduos e permanentes (Longbottom et al.[42], 2008; Haughey et al.[27], 2009; Perfect et al.[57], 2009). No entanto, nenhum trabalho comparou seu desempenho com os parâmetros clínicos empregados na avaliação da atividade de lesões de cárie.

Com o avanço da tecnologia, novos métodos também foram desenvolvidos com a finalidade de auxiliar os métodos convencionais para detectar e quantificar as lesões de cárie. Alguns desses métodos são baseados nos fenômenos ópticos resultantes das diferenças entre as estruturas dentárias hígidas e cariadas, que podem ser quantificadas por

meio de métodos baseados na captação da fluorescência induzida por luz. A fluorescência é o fenômeno no qual a luz é absorvida e reemitida em comprimentos de onda distintos. Essa característica difere o tecido dentário sadio e o cariado quando estimulados por uma luz com comprimento de onda específico (Hibst et al.[29], 2001), podendo ser quantificada e empregada na detecção da lesão de cárie. Dentre os métodos baseados em indução de fluorescência, destacam-se o aparelho QLF (*Quantitative Light-Induced Fluorescence*), a FluoreCam e os aparelhos de fluorescência a laser.

O aparelho QLF (QLF-clin, Inspektor Research Systems BV, Amsterdã, Holanda), disponível comercialmente para o uso clínico, consiste em uma microcâmera intraoral portátil de vídeo CCD colorida, conectada a um computador e a um *software* (QLFpatient, Inspektor Research Systems BV, Amsterdã, Holanda), que possibilitam capturar e analisar as imagens do dente durante o exame clínico. O sistema é constituído por uma lâmpada portátil de xenônio de 50 Watts em forma de arco e um filtro óptico com a finalidade de produzir uma luz azul com comprimento de onda de 370 nm, que é transportada até o dente por meio de um guia de luz. As imagens de fluorescência são filtradas por um filtro de alta passagem ($\lambda \geq 520$ nm) e então capturadas pela microcâmera de vídeo CCD (al-Khateeb et al.[1], 1997). O princípio desse método baseia-se na autofluorescência do esmalte quando estimulado por uma determinada condição de luminosidade. Assim,

o esmalte desmineralizado fluoresce em um comprimento de onda diferente quando comparado ao esmalte hígido. A fluorescência emitida tem relação direta com o conteúdo mineral do esmalte. Para se calcular a perda de fluorescência na lesão de cárie, a fluorescência do tecido hígido que estava originalmente presente no local da lesão, é reconstruída por meio da extrapolação da fluorescência do tecido hígido que se encontra ao redor da lesão de cárie. A diferença entre os valores da lesão e os valores reconstruídos proporciona o cálculo da fluorescência perdida. Assim, essa imagem pode ser utilizada posteriormente para quantificar a área, a profundidade e o volume da lesão de cárie (Zandoná, Zero[69], 2006). Estudos têm mostrado excelente reprodutibilidade do método, além de bons resultados na utilização para detecção de lesões de cárie incipientes (Ando et al.[3], 2001; Kühnisch et al.[41], 2007) e de lesões de cárie secundária (Pretty et al.[58], 2002; González-Cabezas et al.[25], 2003; Ando et al.[2], 2004; Kano-Wilson et al.[33], 2009).

Mais recentemente, um grupo de cientistas norte americanos desenvolveu outro instrumento para detectar, quantificar e monitorar as lesões de cárie baseado na tecnologia da imagem da fluorescência do esmalte (*Fluorescence Enamel Imaging - FEI).* A FluoreCam (Daraza, Corporate Headquarters, Noblesville, Indiana, EUA) consiste em um instrumento portátil acompanhada de um software específico (FluoreCam

Software, Daraza, Corporate Headquarters, Noblesville, Indiana, EUA). O instrumento emite uma luz de alta intensidade que promove a excitação das estruturas dentárias, resultando em uma imagem fluorescente. Esta imagem é capturada e analisada automaticamente pelo software, possibilitando a determinação da perda mineral, além de fornecer informações quanto à área e o volume da mesma. Esse aparelho foi inicialmente avaliado em estudos laboratoriais e clínicos nos EUA como um protótipo denominado PCDS (*Professional Caries Detection System*, Therametrics Technologies, Inc., Indianapolis, IN, EUA). Entretanto, não existem ainda trabalhos publicados avaliando seu desempenho na detecção de lesões de cárie.

O primeiro aparelho baseado em fluorescência a laser, denominado DIAGNOdent 2095 (DD, KaVo, Biberach, Alemanha), foi introduzido no mercado em 1998. Este aparelho emite uma luz laser de diodo (alumínio, gálio, índio e fósforo – AlGaIndP) com comprimento de onda de 655 nm, situado no âmbito vermelho do espectro visível, que é absorvida pelos componentes orgânicos e inorgânicos dos tecidos dentários. O laser diodo chega à superfície dentária através de uma guia luminosa central contida em uma haste óptica flexível. Um fotodetector é capaz de captar a fluorescência emitida pelas porfirinas endógenas (fluoróforos) produzidas pelas bactérias cariogênicas e outros cromóforos presentes no tecido cariado (Hibst et al.[29], 2001). De acordo com o fabricante, essa fluorescência é captada por nove

fibras arranjadas concentricamente à guia luminosa central, quantificada e transformada em valores numéricos que variam de 0 a 99, de acordo com a profundidade da lesão de cárie. Estudos avaliando o DD mostraram boa validade e reprodutibilidade na detecção de lesões de cárie em superfícies oclusais in vitro (Lussi et al.[46], 1999; Shi et al.[63], 2000; Lussi, Francescut[43], 2003; Lussi, Hellwig[45], 2006; Kühnisch et al.[40], 2007; Rodrigues et al.[61], 2008; Rodrigues et al.[62], 2008; Rodrigues et al.[60], 2009; Neuhaus et al.[54], 2010) e in vivo (Lussi et al.[48], 2001; Anttonen et al.[5], 2003; Heinrich-Weltzien et al.[28], 2003; Hamilton et al.[26], 2006; Krause et al.[37], 2007; Diniz et al.[18], 2009). Alguns estudos têm também demonstrado bom desempenho do aparelho na detecção de lesões de cárie secundária (Boston[10], 2003; Ando et al.[2], 2004; Bamzahim et al.[8], 2004; Banzahim et al.[7], 2005).

Em 2005, foi lançado no mercado uma versão mais compacta desse aparelho, denominado DIAGNOdent 2190 ou DIAGNOdent pen (DDpen, KaVo, Biberach, Alemanha), com o objetivo de facilitar o manuseio pelo profissional e melhorar o desempenho para detecção de cárie. Para isso, o fabricante condensou os componentes do aparelho em uma única peça e modificou a estrutura das pontas por fibra de safira. Embora permaneça o mesmo princípio de funcionamento, para o DDpen, tanto o laser diodo quanto a fluorescência emitida pelos tecidos percorrem os mesmos feixes de fibras, mas em sentidos opostos e com comprimentos de onda diferentes

(Lussi, Hellwig[45], 2006; Lussi et al.[44], 2006). Estudos in vitro e in vivo têm mostrado bom desempenho do aparelho na detecção de lesões de cárie oclusais (Lussi, Hellwig[45], 2006; Krause et al.[37], 2007; Huth et al.[30], 2008; Rodrigues et al.[61], 2008) e proximais (Lussi et al.[44], 2006; Braga et al.[13], 2009).

Ainda considerando o fenômeno de fluorescência, outro aparelho foi recentemente desenvolvido. Trata-se da câmera intraoral VistaProof (Dürr Dental, Bietigheim-Bissingen, Alemanha), que ilumina a superfície dentária com luz de comprimento de onda de 405 nm emitida por seis LEDs localizados na sua extremidade, no âmbito azul do espectro visível. O fabricante propõe que essa câmera seja capaz de digitalizar a imagem da superfície dentária no momento da emissão da fluorescência emitida tanto pelos subprodutos bacterianos presentes nas lesões de cárie quanto pelo tecido dentário hígido. Nessas imagens podem ser observadas as diferentes regiões da superfície dentária que fluorescem em verde (aproximadamente com 510nm de comprimento de onda) até o vermelho (aproximadamente com 680nm de comprimento de onda). Para a digitalização e análise das imagens utiliza-se o *software* DBSWIN (Dürr Dental, Bietigheim-Bissingen, Alemanha) que transforma em valores numéricos a relação entre a fluorescência verde e vermelha emitida, representada pela quantidade de pixels das imagens (Thoms[68], 2006). Segundo o fabricante, esses valores (0-

5) estariam relacionados com a extensão da lesão de cárie. No entanto, não existe evidência científica sobre os pontos de corte ideais que devem ser utilizados para determinação da extensão de lesões de cárie em superfícies lisa e oclusal. Estudos in vitro apontam boa reprodutibilidade na detecção de lesões em superfícies lisas e oclusais (Thoms et al.[67], 2007; De Benedetto et al.[17], 2010) e alta sensibilidade para detecção de lesões de cárie oclusal em dentina (Rodrigues et al.[61], 2008).

Outro método para detecção de cárie foi desenvolvido baseado em outros fenônemos ópticos, como a reflexão e a refração da luz, uma vez que a estrutura dentária sadia é geralmente mais translúcida que a desmineralizado, existindo uma diferença nos fenômenos ópticos entre ambas. O Midwest Caries I.D.™ (DENTSPLY Professional, York, PA, EUA) é constituído por uma ponta que emite uma luz LED que atravessa os prismas de esmalte. A reflexão e a refração da luz pelas estruturas dentárias é capturada e convertida em um sinal elétrico por um conjunto de fibras para auxiliar a identificação de lesões de cárie oclusais e proximais em molares e pré molares (Strasser, Sensi[66], 2008). O microprocessador contém um algaritmo computadorizado que determina a presença ou a ausência da lesão por meio de mudanças em translucidez e opacidade ópticas, emitindo dois sinais: um sonoro e um visual. De acordo com o fabricante, a presença de desmineralização ativa propicia a mudança na luz LED verde para a

tonalidade vermelha, com um simultâneo sinal sonoro, que varia de fraco a rápido. Quanto maior a severidade da desmineralização, mais rápido se torna o sinal sonoro emitido. Alguns estudos têm avaliado sua efetividade na detecção de lesões de cárie oclusais e proximais (Braun et al.[15], 2008; Krause et al.[38], 2008) e de lesões de cárie secundária (Kano-Wilson et al.[33], 2009) em dentes permanentes. Embora o Midwest Caries I.D. apresente algumas limitações em sua utilização, é importante que ele seja avaliado nas diferentes situações clínicas.

Apesar dos métodos descritos desempenharem um papel importante como auxiliares no processo de detecção de lesões de cárie, sabe-se que tanto a presença de placa bacteriana quanto de remanescentes de pastas e/ou pó profiláticos podem influenciar negativamente em seus desempenhos (Lussi et al.[46], 1999; Mendes et al.[51], 2004; Anttonen et al.[6], 2005; Lussi, Reich[49], 2005). Para a realização de um correto exame, a limpeza da superfície dentária é um procedimento indispensável para a remoção da placa bacteriana, facilitando a correta detecção das lesões de cárie quando são utilizados os método baseados na captação da fluorescência emitida por luz (Mendes et al.[51], 2004; Lussi et al.[47], 2005; Lussi, Reich[49], 2005; Strasser, Sensi[66], 2008). Na literatura não foi encontrada nenhuma pesquisa avaliando a influência dos meios de profilaxia profissional sobre o desempenho do DIAGNOdent pen e da câmera VistaProof. Assim, torna-se importante o

conhecimento por parte dos profissionais sobre a influência dos produtos profiláticos nos resultados obtidos com os métodos baseados na captação da fluorescência emitida por luz, uma vez que falsos resultados poderão afetar a decisão de tratamento.

É importante ressaltar que o desempenho clínico do DIAGNOdent, do DIAGNOdent pen e da câmara VistaProof está relacionando com os pontos de corte empregados na prática odontológica. Diferentes pontos de corte vêm sendo propostos para o DIAGNOdent, tanto pelo fabricante, como pelos estudos in vitro (Lussi et al.[46], 1999; Lussi, Hellwig[45], 2006; Rodrigues et al.[61], 2008) e in vivo (Lussi et al.[48], 2001; Diniz et al.[18], 2009). Até o presente momento, apenas dois estudos in vivo propuseram pontos de corte e avaliaram o desempenho do DIAGNOdent pen na detecção de lesões de cárie oclusal (Krause et al.[37], 2007; Huth et al.[30], 2008). Entretanto, nenhum ponto de corte clínico foi descrito na literatura para a câmera VistaProof. Além disso, observa-se nos estudos in vivo a privação na validação de superfícies oclusais hígidas e com lesões de cárie em esmalte por meio de intervenção operatória devido às questões éticas envolvidas em uma situação clínica (Anttonen et al.[5], 2003; Heinrich-Weltzien et al.[28], 2003; Hamilton et al.[26], 2006; Krause et al.[37], 2007). Assim, novos estudos clínicos utilizando dentes indicados para extração com posterior validação histológica

são necessários para estabelecer os pontos de corte mais apropriados para cada método.

Faz-se necessária também a validação clínica do critério visual ICDAS para a detecção de lesões de cárie primária em dentes decíduos e permanentes. Além disso, a literatura carece de estudos a respeito do critério LLA associado ao ICDAS e do novo método baseado em luminescência para avaliação da atividade de lesões de cárie.

Alguns métodos também vêm sendo avaliados como auxiliares na detecção de cárie secundária ao redor de restaurações de amálgama e de resina composta, como o aparelho QLF (González-Cabezas et al.[25], 2003; Ando et al.[2], 2004), a fluorescência a laser (Boston[10], 2003; Ando et al.[2], 2004; Bamzahim et al.[8], 2004; Banzahim et al.[7], 2005; Braga et al.[11], 2010) e o Midwest Caries I.D.™ (Kano-Wilson et al.[33], 2009). Embora algumas pesquisas apontem bons resultados, ainda existem controvérsias com relação ao desempenho desses métodos.

Diante desses resultados, os questionamentos relacionados à real efetividade desses métodos ainda permanecem em aberto. Dessa forma, faz-se necessário a realização de estudos adicionais com o objetivo de avaliar o desempenho do critério visual ICDAS e dos novos métodos para detecção e avaliação da atividade de lesões de cárie primária e secundária em superfícies oclusais. Além disso, é de extrema importância que esses

estudos proporcionem ao profissional o conhecimento da técnica e segurança no emprego desses métodos na prática clínica diária como uma forma auxiliar aos métodos tradicionais já existentes.

PROPOSIÇÃO

Proposição Geral

Avaliar a efetividade de métodos empregados na detecção e na avaliação da atividade de lesões de cárie primária e secundária em superfícies oclusais de dentes decíduos e permanentes.

Proposição Específica

Capítulo 1. (A) Avaliar in vitro a influência de métodos de profilaxia profissional nas medidas de fluorescência e (B) avaliar a influência desses métodos no desempenho do DIAGNOdent, do DIAGNOdent *pen* e da câmera VistaProof na detecção de lesões de cárie oclusal em dentes permanentes.

Capítulo 2. (A) Determinar os pontos de corte clínicos ideais para o DIAGNOdent, o DIAGNOdent *pen* e a câmera VistaProof e (B) avaliar a validade clínica de métodos convencionais e de métodos baseados na captação da fluorescência induzida por luz na detecção de lesões de cárie oclusal em dentes permanentes utilizando o padrão ouro histológico para total validação da amostra.

Capítulo 3. (A) Comparar in vivo o critério ICDAS-LAA e o critério clínico visual/táctil para avaliação de atividade de lesões de cárie, (B) comparar os resultados in vivo de ambos os critérios com os resultados in vitro obtidos pelo novo método de luminescência para avaliação de atividade de lesões de cárie e (C) comparar os dois critérios com o método de luminescência para determinar o status da atividade de lesões de cárie em molares decíduos reexaminados após 2 meses de acompanhamento.

Capítulo 4. (A) Avaliar o desempenho do critério visual ICDAS, do DIAGNOdent, do Midwest Caries I.D., do QLF e do PCDS na detecção de lesões naturais de cárie ao redor de restaurações de amálgama e de resina composta em dentes permanentes e (B) determinar a relação entre a presença da lesão de cárie secundária e a presença/ausência de diferentes tamanhos de defeitos marginais.

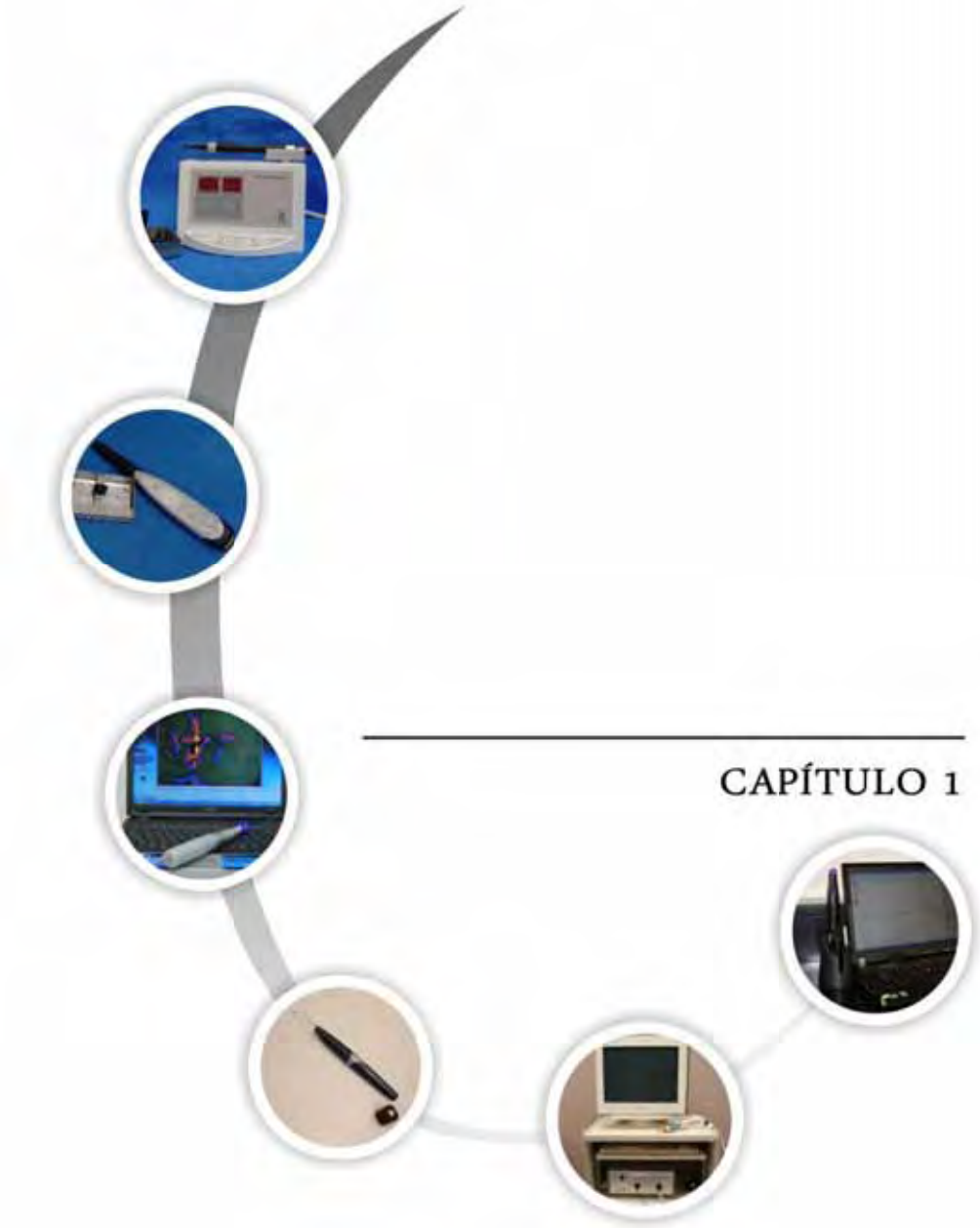
Esta tese será apresentada na forma de 4 capítulos (artigos) intitulados:

Capítulo 1: "Influence of different professional prophylaxis methods on fluorescence measurements for detection of occlusal caries" - Submetido para publicação na revista *Caries Research.*

Capítulo 2: "Validity of conventional and fluorescence-based methods for occlusal caries detection: an in vivo study with histological validation" - Submetido para publicação na revista *Caries Research.*

Capítulo 3: ***"Evaluation of occlusal caries activity using two clinical assessment systems and a novel luminescence assay in primary teeth"*** **-** Será submetido para publicação na revista *Journal of Dental Research.*

Capítulo 4: "Evaluation of ICDAS and new technologies for detecting caries around amalgam and composite resin restorations" - Será submetido para publicação na revista *Operative Dentistry.*



CAPÍTULO 1

Title: Influence of different professional prophylaxis methods on fluorescence measurements for detection of occlusal caries

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Short title: Prophylaxis and fluorescence

Key words: Occlusal caries, Caries detection, Fluorescence, Prophylaxis paste, Professional prophylaxis, Permanent teeth

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Declaration of interests

The undersigned authors declare that they have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that might introduce bias or affect their judgment or that could be construed as influencing the position presented herein or the review of the manuscript entitled "*Influence of professional prophylaxis methods on fluorescence measurements for detection of occlusal caries detection".*

Abstract

This in vitro study aimed to evaluate the effects of professional prophylaxis procedures on fluorescence measurements and on DIAGNOdent 2095 (LF), DIAGNOdent 2190 (LFpen) and VistaProof camera (FC) performance in detecting occlusal caries in permanent teeth. All assessments were performed in 110 permanent teeth by two examiners at (I) baseline, (II) after professional prophylaxis and rinsing, and (III) after a second rinsing. After baseline, the teeth were randomly divided according to the professional prophylaxis used: sodium bicarbonate jet (group A) or prophylaxis paste (group B). Teeth were analysed for extension of caries. There was an increase in fluorescence measurements across the conditions for both groups. Statistical differences were observed for LF and LFpen between both groups in conditions II and III. Considering sensitivity, specificity and accuracy, at D_1 threshold, there was no significant difference in group A among the conditions. At D_3, all methods were similar. For group B, at D_1 threshold, sensitivity increased significantly in conditions II and III for LF and LFpen. At D_3, LF and LFpen showed higher sensitivity. It can be concluded that the choice of professional prophylaxis method can significantly influence the fluorescence measurements and the performance of fluorescence-based methods for detection of occlusal caries.

Introduction

Early caries detection is a challenge in dentistry due to changes in carious lesions' behaviour in recent decades. However, the slower progression of caries offers the opportunity for dental practioners to detect and manage caries before irreversible destruction of the tooth takes place [Ferreira Zandoná and Zero, 2006]. For this reason, the importance of early caries detection has grown in recent years [Sheehy et al., 2001].

Several new methods to detect and quantify early carious lesions and the level of mineral loss on smooth and occlusal surfaces have been developed and proposed [Mendes et al., 2006]. Fluorescence can be used for caries detection because of the variation in fluorescence observed between sound and demineralised dental tissue [Hibst et al., 2001; Bader and Shugars, 2004].

The first laser fluorescence device (LF - DIAGNOdent 2095, KaVo, Biberach, Germany) was developed in 1998. It is able to capture the fluorescence emitted from bacterial porphyrins and other chromophores when the teeth are stimulated by its diode laser at a wavelength of 655 nm [Shi et al., 2000; Hibst et al., 2001]. The changes in the fluorescence intensity are numerically quantified and translated to values ranging from 0 to 99 [Lussi et al., 1999; Lussi, Hellwig, 2006]. In 2005, a new laser fluorescence device was introduced (LFpen - DIAGNOdent 2190, KaVo, Biberach, Germany) and has

been used to detect occlusal and approximal carious lesions [Lussi and Hellwig, 2006; Lussi et al., 2006]. This device is a handheld laser caries detection aid similar to the previous LF.

The fluorescence camera device (FC - VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) has recently been devised and is able to emit blue light at 405 nm wavelength and capture and digitalise images from the teeth while they are emitting fluorescence [Rodrigues et al., 2008a]. Custom software converts the relation between green and red fluorescence to numerical values according to the pixel numbers in each image [Thoms, 2006]. However, only two published studies suggest that this new system has the ability to detect occlusal caries in permanent and primary teeth with good validity and reproducibility [Rodrigues et al., 2008a; De Benedetto et al., 2010].

Although some studies have shown that LF, LFpen and FC present good validity and reproducibility for occlusal caries detection [Lussi et al., 2001; Lussi and Hellwig, 2006; Rodrigues et al., 2008a; Diniz et al., 2009; De Benedetto et al., 2010], it is important to stress that dental plaque and remnants of material such as pastes, powders or gels from the cleaning procedure may emit some fluorescence and lead to false positive results [Lussi et al., 1999; Hosoya et al., 2004; Mendes et al., 2004; Anttonen et al., 2005; Lussi and Reich, 2005]. According to Mendes et al. [2004], artificial

dental plaque might reduce LF values. Thus, professional cleaning and drying is advised to ensure the correct detection of caries lesions through fluorescence measurements [Neuhaus et al., 2009]. To our knowledge, the influence of professional prophylaxis procedures on the LFpen and FC devices has not been yet evaluated. Because prophylaxis materials can influence fluorescence measurements, it is important to point out that these systems may under- or overscore carious lesions' severity, which may interfere with treatment planning.

Therefore, the aims of this in vitro study were (1) to evaluate the influence of professional prophylaxis procedures (sodium bicarbonate jet and prophylaxis paste) on fluorescence measurements and (2) to evaluate the influence of these procedures on the LF, LFpen and FC performance in detecting occlusal caries in permanent teeth.

Materials and Methods

The research protocol was approved by the Local Ethics Committee in Araraquara, São Paulo, Brazil (Protocol 04/08). One hundred and ten extracted third permanent human molars from sound to carious were selected from a pool of teeth that were stored at - 20°C until use. An earlier study showed that this method of storage does not significantly change the infrared fluorescence measurements [Francescut et al., 2006]. All teeth were

extracted by dental practitioners in Brazil because of periodontal disease or for orthodontic reasons. Prior to extraction, the patients were informed about the use of their teeth for research purposes, and their written consent was obtained. The teeth were defrosted for 3 hours, and the calculus and debris were removed using a scaler. The teeth were then cleaned for 15 s with water and a toothbrush (Colgate Professional Extra Clean, Colgate-Palmolive, São Paulo, Brazil) [Lussi and Reich, 2005]. During the experiment, the teeth were stored individually under 100% humidity at - 20°C.

The occlusal surfaces were photographed at 10x magnification using a stereomicroscope (SZX7, Olympus, Tokyo, Japan). One occlusal site per tooth was selected and marked on the photograph with a dot to allow the examiners to localise the test site precisely during the exams. All assessments were independently carried out by two experienced examiners using the following devices: DIAGNOdent 2095 (LF - KaVo, Biberach, Germany), DIAGNOdent 2190 (LFpen – KaVo, Biberach, Germany) and VistaProof fluorescence camera (FC – Dürr Dental, Bietigheim-Bissingen, Germany). The examinations were performed in three conditions: (I) before professional prophylaxis (baseline), (II) after professional prophylaxis for 10 sec, rinsing off for 3 sec and drying for 3 sec, and (III) after a second rinsing off for 3 sec and drying for 3 sec.

The LF and LFpen measurements were performed using probe tip “A” and the cylindrical sapphire fibre tip, respectively, according to the manufacturer’s instructions. Before each measurement, the devices were calibrated with a ceramic standard, and the zero value of fluorescence of a sound part of the cuspal area of the buccal surface was recorded. The tip was placed on the selected site and rotated around the vertical axis until the highest fluorescence reading was obtained. The peak values were recorded, and the zero value of fluorescence was subtracted [Rodrigues et al., 2008a].

The FC measurements were performed in a dark environment, using a black box to simulate illumination of the oral environment and the thicker spacer, which provides a distance of 1.0 cm from the tooth [De Benedetto et al., 2010]. The images were analysed by FC-specific software (DBSWIN, Dürr Dental, Bietigheim-Bissingen, Germany) that translates the rates of red and green fluorescence to numbers. This software shows the regions of the teeth that emit fluorescence varying from green (approximately 510 nm wavelength) to red (approximately 680 nm wavelength) and values corresponding to the lesion severity [Rodrigues et al., 2008a]. The values were recorded for further analysis.

After the baseline measurements, the teeth were then randomly divided according to the type of professional prophylaxis. An attempt was

made to form groups with similar numbers of sound and carious teeth by visual inspection. The groups were arranged as follows:

- Group A (n = 55) - PROFI III BIOS® (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) using sodium bicarbonate powder and water;
- Group B (n = 55) - Odahcam® prophylaxis paste (Dentsply, Petrópolis, Rio de Janeiro, Brazil) using a slow-rotating contra-angle handpiece with a Robinson brush.

After the prophylaxis procedure, teeth were rinsed off for 3 sec and dried for 3 sec with a 3-in-1 syringe (II). Then the fluorescence values were recorded again. New measurements were taken after again rinsing off for 3 sec and drying for 3 sec (III) [Lussi and Reich, 2005].

LF and LFpen values of both prophylaxis materials were tested. The first layer of each product was discarded. The products were placed on a piece of glass, and the values were obtained by putting the tips in close contact with both materials. The tips were cleaned with ethanol (100%) and the procedure repeated. The resulting values were recorded

For validation, the teeth were sectioned longitudinally perpendicular to the test site with a water-cooled diamond blade (ISOMET 1000, Buehler Ltda., Lake Bluff, II, USA). Then they were ground using silicon carbide paper with decreasing grain sizes of 400, 600, 1200 and 2000. The progression of

the grinding process was constantly checked under the stereomicroscope (magnification 10x) until the periphery of the site was reached. The tooth surfaces were then coloured with saturated rhodamine B (Fluka, Buch, Switzerland), and the histological examination was performed according to the rhodamine B penetration either into the enamel or into both enamel and dentine tissues. The sites were assessed for caries extension (magnification 10x) according to Lussi et al. [1999]: caries free (0), caries extending up to halfway through the enamel (1), caries extending into the inner half of enamel (2), caries in dentine (3) and deep dentine caries (4).

Statistical Analysis

For each method, the data from both examiners in each phase were combined and analysed by descriptive methods using histograms and QQ plots (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA). As they were not normally distributed, the Wilcoxon test for paired data was used to compare the fluorescence measurements among the three phases. A Mann-Whitney U test was applied to compare the fluorescence values between the two groups during different phases. The level of significance for all tests was chosen as $p<0.05$.

The optimal cut-off limits for LF, LFpen and FC were determined by the highest sum of sensitivity and specificity at each threshold. The baseline

measurements from both examiners were used for each method. Sensitivity, specificity, accuracy and area under the ROC curve (A_z) were calculated in each condition at D_1 (considering as disease gold-standard scores from 1 to 4) and D_3 (considering as disease gold-standard scores of 3 and 4) diagnostic thresholds (MedCalc for Windows, version 9.3.0.0, Mariakerke, Belgium). The McNemar test ($p<0.05$) was applied to compare the performance in the different conditions. In addition, a nonparametric statistical test was applied to assess the difference in areas under the ROC curves (A_z) [Hanley and McNeil, 1983].

Intra-class correlation (ICC) was used to assess inter-examiner reproducibility in the three phases for each group. The ICC was assessed as poor when the values were below 0.40, fair for values between 0.40 and 0.59, good for values between 0.60 and 0.75, and excellent for values above 0.75. ICC values above 0.75 denoted excellent agreement, while values between 0.40 and 0.75 indicated good agreement [Fleiss, 1981].

Results

Of the 55 occlusal test sites analysed in group A, the histological examination showed that 2 were caries free, 8 had caries extending up to halfway through the enamel, 34 had caries extending to the inner half of enamel, 10 had caries in dentine and 1 had deep dentinal caries. For group B

(n=55), 6 were caries free, 9 had caries extending up to halfway through the enamel, 34 had caries extending to the inner half of enamel, 4 had caries in dentine and 2 had deep dentinal caries.

Tables 1 and 2 show an overview of the LF, LFpen and FC measurements at baseline (I), after professional prophylaxis for 10 s, rinsing off for 3 s and drying for 3 s (II), and after a second rinsing off for 3 s and drying for 3 s (III) for groups A and B, respectively. For group A, statistically significant increases were observed in measurements made by LF between conditions II and III, by LFpen among all conditions and by FC between conditions I and III. For group B, significant increases were demonstrated for LF, LFpen and FC between conditions I and II and conditions I and III.

The measurements performed under different prophylaxis procedures are shown in table 3. For the LF and LFpen measurements, statistically significant differences were observed between conditions II and III. For FC measurements, there is no significant difference among all conditions.

The measurements of the prophylaxis materials alone revealed LF and LFpen values of up to 99. These maximum values were reached by the Odahcam® prophylaxis paste. The sodium bicarbonate powder showed an inherent fluorescence of 6.

The optimal cut-off limits for the LF, LFpen and FC devices, determined by the point in the ROC curve at which the sum of sensitivity and specificity is maximised, are shown in table 4.

Specificity, sensitivity, accuracy and area under the ROC curve (A_z) at the D_1 and D_3 thresholds are presented in tables 5 and 6 for groups A and B, respectively. For group A, at the D_1 threshold, there was no significant difference among the conditions for all methods. The LFpen presented a good balance between sensitivity and specificity values. In general, all methods presented similar performance at the D_3 threshold. Significant decreases in specificity and accuracy were found for the LFpen between conditions I and II and conditions I and III; however, no difference was observed between conditions II and III. For group B, at the D_1 threshold, the sensitivity increased significantly between conditions I and II and conditions I and III for both LF and LFpen. FC showed an excellent balance between sensitivity and specificity, and the largest areas under the ROC curves. At the D_3 threshold, LF and LFpen showed higher sensitivities between the conditions and a significant decrease in specificity and accuracy values between conditions I and II and conditions I and III. FC showed lower sensitivity values and higher specificity and accuracy values, with differences between conditions I and III.

Table 7 represents the reproducibility assessed by calculating the intra-class correlation. The ICCs for inter-examiner reproducibility values ranged from 0.83 to 0.94 (LF), 0.78 to 0.91 (LFpen) and 0.85 to 0.94 (FC), indicating excellent agreement between the examiners.

Discussion

It is well known that teeth should be cleaned and dried before visual examination to achieve accurate caries detection. Adjunct methods must be used for a second opinion in questionable cases. However, cleaning procedures could leave remnants of cleaning materials in small and narrow areas, such as pits and fissures on occlusal surfaces, which might influence fluorescence measurements, resulting in an incorrect diagnosis [Lussi and Reich, 2005].

Researchers have used different methods of prophylaxis before LF examination, such as a toothbrush and fluoride-free pumice [Lussi et al., 1999], explorer [Lussi et al., 2001], single-tufted toothbrush and water [Sheehy et al., 2001], airflow device [Heinrich-Weltzien et al., 2003], rubber cup and polishing paste [Anttonen et al., 2005], sodium bicarbonate jet [Lussi and Reich, 2005; Diniz et al., 2008; Rodrigues et al., 2008a], aluminium oxide jet [Rodrigues et al., 2008b] and rubber cup and pumice slurry [Diniz et al., 2009]. In this study, the two most common prophylaxis procedures used for

effective bacterial plaque removal on the various dental surfaces were chosen. Both methods (prophylaxis paste and sodium bicarbonate jet) were applied for 10 seconds, as described by Honório et al. [2006]. According to Lanza et al. [2000], sodium bicarbonate is able to clean both soft and hard tartar from the teeth without any harm to the enamel while diminishing the amount of pathogenic microflora in the oral cavity. The prophylaxis paste is excellent for stain removal and teeth polishing.

Concerning the fluorescence measurements in the three phases of this study, there was a gradual increase after both prophylaxis procedures and after the second rinsing off and drying when compared to the baseline measurements. Anttonen et al. [2005] also observed an increase in LF values after professional cleaning. Contrary to this result, another study showed that cleaning sound surfaces with prophylaxis paste or powder did not significantly interfere with LF reading when the tooth surface was rinsed thoroughly for 3 s with a 3-in-1 syringe [Lussi, and Reich, 2005]. According to Mendes et al. [2004], the presence of plaque would yield poor LF results. These authors suggested that the plaque could act as a barrier to the light from the fluorescence devices. However, other studies observed higher LF values in the presence of plaque [Sultanov, 2001; Bamzahim et al., 2002]. In our study, the increase in values after prophylaxis could be explained by the debris barrier removal from the occlusal surfaces or by the short duration of rinsing

off to remove the prophylaxis materials remnants. Furthermore, it is noteworthy that LF and LFpen may provide different fluorescence measurements because some factors can influence the results, such as the examiner's experience with using the devices.

The manufacturer of LF device advises that the prophylaxis materials should be non-fluorescent [Anttonen et al., 2005]. However, according to Lussi and Reich [2005], the industry has continued to formulate prophylaxis pastes with fluorescent materials. It is important to point out that LF and LFpen measurements are statistically significantly higher when using the prophylaxis paste than when using the sodium bicarbonate jet. This fact could be attributed to the porosity of carious tissue, which could lead to some penetration of the prophylaxis material, and to the higher inherent fluorescence value of this paste (i.e., 99). This light blue paste is formulated with pumice powder (abrasive), calcium carbonate, glycerin, water, sorbitol, dye, fruit flavouring, thickener and preservatives. According to Hosoya et al. [2004], tooth-polishing pumice-containing pastes showed high fluorescence values and should not be used for tooth cleaning before LF measurements. The contents of the prophylaxis paste used in our study, especially pumice, might have influenced the fluorescence measurements and given a false fluorescence measurement by LF and LFpen. On the other hand, sodium bicarbonate powder showed a low inherent fluorescence value of 6. This fact

could be due to its white colour, pH and composition of a fine solid crystalline powder. Lussi and Reich [2005] also observed high fluorescence values (i.e., 99) for some prophylaxis pastes, such as Nupro mint medium, Nupro cherry medium and Nupro orange fine (Dentsply De Trey, Konstanz, Germany); and lower fluorescence values (9) for Prophyflex powder (KaVo, Biberach, Germany).

Regarding FC measurements, no difference between the two prophylaxis methods could be observed. This outcome is very notable in view of the fact that, for this method, the prophylaxis procedure used before image capturing does not matter. However, it can be speculated that the amount of the prophylaxis materials used in this study may not be strong enough to become detectable in the FC image. It would be interesting to investigate the fluorescence spectra of these prophylaxis materials. Thus, as FC examination is performed on the tooth indirectly, it could be suggested that some factors may influence the quality of the FC images, such as the anatomical structures of occlusal surfaces and the complex light scattering pattern generated, which can make the reconstruction of the fluorescence values difficult. In a different way, LF and LFpen measurements directly capture the fluorescence emitted by dental tissues.

Considering the sodium bicarbonate jet, sensitivity, specificity, accuracy and area under the ROC curve values did not vary significantly

among the different conditions for all methods at the D_1 threshold. However, LFpen and FC showed superior results in terms of sensitivity, as did LF and LFpen in terms of specificity. A small increase in sensitivity and accuracy values after the second rinsing and drying procedure could also be observed. If the teeth are not rinsed vigorously with a water spray, there could be a high chance of interference with fluorescence measurements [Lussi and Reich, 2005]. However, higher sensitivity and lower specificity values were observed for LF and LFpen by Lussi and Hellwig [2006]. In that study, the teeth were also cleaned by sodium bicarbonate jet before fluorescence assessments, although their aim was not to investigate the impact of prophylaxis treatment on laser fluorescence performance. The difference found could be attributed to the different cut-off limits used in their study, which were lower for enamel caries for both devices when compared to ours. If their cut-offs were used in our study, higher sensitivity and accuracy values would be achieved. At the D_3 threshold, LF and LFpen presented similar performance in the three conditions. For LFpen, there was a significant decrease in the specificity and accuracy values between baseline and following the prophylaxis procedure and between baseline and following the second rinsing off and drying. Rodrigues et al. [2008a], after cleaning the teeth with a sodium bicarbonate jet, also observed lower values of specificity and accuracy for LFpen. The areas under the ROC curve were smaller and did not change between

conditions. The lower sensitivity values observed for all methods could be explained by the small number of teeth with caries in dentin (20%) and the cut-off used for dentin caries (>34). Lower sensitivity was also found by Rodrigues et al. [2008a] for LF, but in their study, 46% of the teeth had dentin caries and the cut-off for dentin caries was >17. In contrast to this finding, higher values of sensitivity and specificity were described for LF and LFpen in an in vitro study for occlusal caries detection [Lussi and Hellwig, 2006].

Concerning the performance of the methods for the prophylaxis paste group (group B), at D_1 threshold, the sensitivity values increased significantly for LF and LFpen after the cleaning procedure and after the second rinsing off and drying procedure. According to Honório et al. [2006], the prophylaxis procedure with pumice slurry may be very harmful to a demineralised tooth by rupturing the surface layer of white spot lesion, exposing the lesion body. In the present study, although the surface layer was not evaluated, the increase in the sensitivity values might be due to wear to the demineralised surface. This fact can be attributed to the pumice present in the prophylaxis paste used, which improved the LF and LFpen performance. Nevertheless, FC showed an excellent balance between sensitivity and specificity and the largest areas under the ROC curves, with no significant difference among the three conditions analysed in this study. De Benedetto et al. [2010] state that as FC probably exhibits similar behaviour to the LF device, similar results

would be expected when detecting increases in fluorescence due to the presence of bacterial metabolites. However, this finding was not observed in our study. At D_3 threshold, LF and LFpen showed higher sensitivity values, with no statistically significant difference among the three conditions. This fact can be attributed to the increased values of LF and LFpen measurements after the prophylaxis and after the second rinsing and drying procedure. On the other hand, a significant decrease in specificity and accuracy values after prophylaxis and after the second rinsing and drying procedure was also observed. It is important to stress that in this group, only 11% of the teeth had dentin caries lesions, which probably influenced our results. FC presented lower sensitivity values and higher specificity and accuracy values. A recent study evaluated the LF, LFpen and FC measurements on occlusal surfaces after cleaning the teeth with a rotating brush and pumice/water slurry and rinsing to remove remnants from the fissures [De Benedetto et al., 2010]. However, this study did not evaluate the validity of these methods, which hinders the comparison with our results.

In relation to interexaminer reproducibility, high ICC values were found for the fluorescence-based methods and were not significantly different among the three conditions evaluated in this study. Lussi and Hellwig [2006], Diniz et al. [2008] and Rodrigues et al. [2008a] also described high ICC values for LF and LFpen in detecting carious lesions in occlusal surfaces in

vitro. For FC, similar ICC values were also reported in previous studies [Rodrigues et al., 2008a; De Benedetto et al., 2010]. The excellent reproducibility presented in all phases shows that similar results can be found over different assessments and conditions.

In conclusion, the present results indicate that different professional prophylaxis methods can significantly influence fluorescence measurements and the performance of fluorescence-based methods for occlusal caries detection. Regarding the professional prophylaxis procedures, the fluorescence-based methods presented higher fluorescence measurements in the prophylaxis paste group than in the sodium bicarbonate jet group. Thus, the performance in terms of sensitivity was improved after careful rinsing off of the occlusal surfaces when the prophylaxis paste was used. However, the professional prophylaxis using the sodium bicarbonate jet did not significantly influence the performance of the fluorescence-based methods. Despite the limitations of this in vitro study, such as the small numbers of sound teeth and teeth with dentin caries, it can be concluded that FC is less influenced by the choice of prophylaxis procedure than are LF and LFpen. It also underscores the necessity to rinse the teeth with water spray after prophylaxis procedures to eliminate any remnants, especially of prophylaxis paste, in pits and fissures. Special care is recommended when using LF, LFpen and FC after professional cleaning as the potential for

obtaining incorrect results exists. Although new caries detection methods are emerging, fluorescence-based methods should be used to obtain a second opinion for caries detection. Further studies, mainly in vivo, should be performed to evaluate the possible influence of prophylaxis materials used in dental practice on fluorescence measurements.

Acknowledgments

The authors are grateful to Walter B. Buergin (School of Dental Medicine, University of Bern, Switzerland) for assistance with the statistical analysis. In addition, the authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for their financial support. Michele Baffi Diniz submitted this study to the School of Dentistry of Araraquara (UNESP - Univ. Estadual Paulista, Brazil) as a requirement for the PhD degree in Paediatric Dentistry.

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Table 1: LF, LFpen and FC measurements for group A (n=55) in the three conditions (mean ± SD).

Condition	Mean ± SD		
	LF	LFpen	FC
I	22.1 ± 22.0[a,b]	31.2 ± 28.0[a]	1.5 ± 0.4[a]
II	23.2 ± 25.7[a]	36.0 ± 32.1[b]	1.5 ± 0.4[a,b]
III	25.0 ± 26.8[b]	38.4 ± 32.7[c]	1.6 ± 0.5[b]
Significant differences are represented by different superscript letters in a single column (Wilcoxon test, $p<0.05$)			

Table 2: LF, LFpen and FC measurements for group B (n=55) in the three conditions (mean ± SD).

Condition	Mean ± SD		
	LF	LFpen	FC
I	21.5 ± 24.8[a]	28.8 ± 27.7[a]	1.4 ± 0.3[a]
II	35.8 ± 32.0[b]	44.5 ± 33.9[b]	1.5 ± 0.4[b]
III	36.4 ± 32.0[b]	43.1 ± 34.3[b]	1.5 ± 0.4[b]
Significant differences are represented by different superscript letters in a single column (Wilcoxon test, $p<0.05$)			

Table 3: Comparison of LF, LFpen and FC means ± SD values between the two groups in the three conditions.

Group	LF Mean ± SD		
	I	II	III
A	22.1 ± 22.0[a]	23.2 ± 25.7[a]	25.0 ± 26.8[a]
B	21.5 ± 24.8[a]	35.8 ± 32.0[b]	36.4 ± 32.0[b]
Group	LFpen Mean ± SD		
	I	II	III
A	31.2 ± 28.0[a]	36.0 ± 32.1[a]	38.4 ± 32.7[a]
B	28.8 ± 27.7[a]	44.5 ± 33.9[b]	43.1 ± 34.3[b]
Group	FC Mean ± SD		
	I	II	III
A	1.5 ± 0.4[a]	1.5 ± 0.4[a]	1.6 ± 0.5[a]
B	1.4 ± 0.3[a]	1.5 ± 0.4[a]	1.5 ± 0.4[a]

Significant differences are represented by different superscript letters in a single column (Mann-Whitney test, $p<0.05$).

Table 4: Optimal cut-offs for LF, LFpen and FC.

Histology	Lesion depth	LF	LFpen	FC
0	Sound	0-15	0-10	0-1.1
1,2	Enamel caries	16-25	11-34	1.2-1.7
3,4	Dentine caries	>25	>34	>1.7

Table 5: Sensitivity (Se), specificity (Sp), accuracy (Ac) and area under the ROC curve (A_z) for LF, LFpen and FC for group A (sodium bicarbonate jet) in all conditions.

Condition	LF							
	D_1				D_3			
	Se	Sp	Ac	A_z	Se	Sp	Ac	A_z
I	0.50[a]	1.00[a]	0.52[a]	0.86[a]	0.50[a]	0.73[a]	0.68[a]	0.64[a]
II	0.51[a]	1.00[a]	0.53[a]	0.80[a]	0.50[a]	0.76[a]	0.71[a]	0.60[a]
III	0.57[a]	1.00[a]	0.58[a]	0.86[a]	0.45[a]	0.72[a]	0.66[a]	0.57[a]
Condition	LFpen							
	D_1				D_3			
	Se	Sp	Ac	A_z	Se	Sp	Ac	A_z
I	0.75[a]	1.00[a]	0.75[a]	0.85[a]	0.45[a]	0.74[a]	0.68[a]	0.62[a]
II	0.68[a]	1.00[a]	0.69[a]	0.81[a]	0.50[a]	0.60[b]	0.58[b]	0.56[a]
III	0.70[a]	0.75[a]	0.70[a]	0.83[a]	0.50[a]	0.55[b]	0.54[b]	0.56[a]
Condition	FC							
	D_1				D_3			
	Se	Sp	Ac	Az	Se	Sp	Ac	Az
I	0.81[a]	0.50[a]	0.80[a]	0.73[a]	0.55[a]	0.81[a]	0.75[a]	0.65[a]
II	0.77[a]	0.50[a]	0.76[a]	0.76[a]	0.55[a]	0.74[a]	0.70[a]	0.65[a]
III	0.79[a]	0.50[a]	0.78[a]	0.76[a]	0.50[a]	0.73[a]	0.68[a]	0.66[a]

D_1: 0=sound; 1-4=decayed
D_3: 0-2=sound; 3-4=decayed
Significant differences are represented by different superscript letters in a single column.
(McNemar test, $p<0.05$ for Se, Sp and Ac; nonparametric statistical test for A_z)

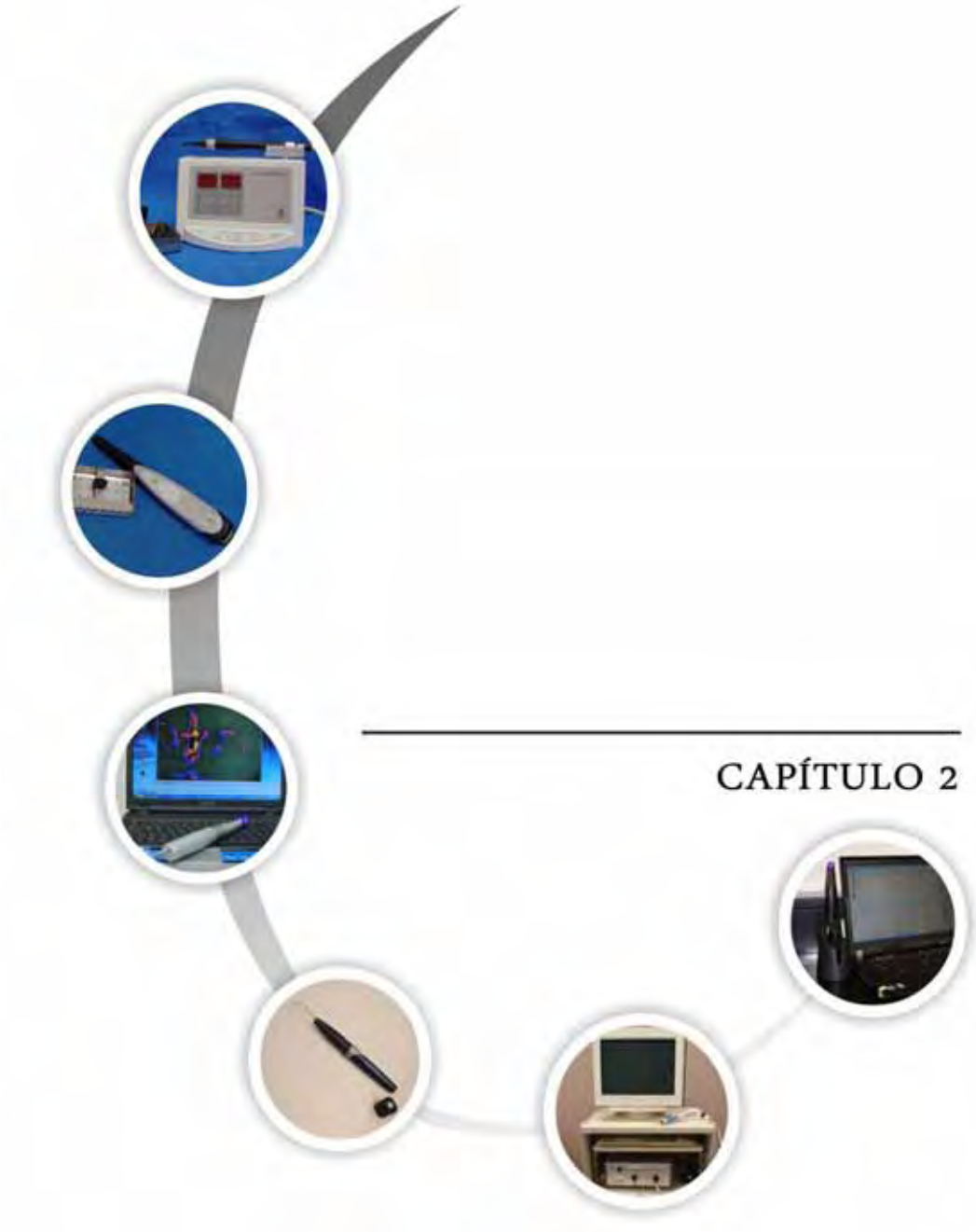
Table 6: Sensitivity (Se), specificity (Sp), accuracy (Ac) and area under the ROC curve (A_z) for LF, LFpen and FC for group B (prophylaxis paste) in all conditions.

Condition	LF							
	D_1				D_3			
	Se	Sp	Ac	A_z	Se	Sp	Ac	A_z
I	0.42^a	0.92^a	0.47^a	0.63^a	1.00^a	0.81^a	0.82^a	0.89^a
II	0.64^b	0.58^b	0.64^b	0.69^a	0.75^a	0.58^b	0.60^b	0.61^b
III	0.69^b	0.50^b	0.67^b	0.72^a	0.83^a	0.56^b	0.59^b	0.70^b
Condition	LFpen							
	D_1				D_3			
	Se	Sp	Ac	A_z	Se	Sp	Ac	A_z
I	0.71^a	0.58^a	0.70^a	0.66^a	0.92^a	0.77^a	0.78^a	0.84^a
II	0.83^b	0.42^a	$0.78^{a,b}$	0.71^a	0.75^a	0.52^b	0.55^b	0.65^b
III	0.86^b	0.50^a	0.82^b	0.76^a	0.83^a	0.59^b	0.62^b	$0.71^{a,b}$
Condition	FC							
	D_1				D_3			
	Se	Sp	Ac	Az	Se	Sp	Ac	Az
I	0.76^a	1.00^a	0.78^a	0.87^a	0.58^a	0.89^a	0.85^a	0.84^a
II	0.77^a	0.92^a	0.78^a	0.86^a	0.67^a	$0.81^{a,b}$	$0.79^{a,b}$	0.80^a
III	0.81^a	0.92^a	0.82^a	0.90^a	0.58^a	0.76^b	0.74^b	0.75^a

D_1: 0=sound; 1-4=decayed
D_3: 0-2=sound; 3-4=decayed
Significant differences are represented by different superscript letters in a single column.
(McNemar test, $p<0.05$ for Se, Sp and Ac; nonparametric statistical test for A_z)

Table 7: Intra-class correlation coefficients (ICC) for inter-examiner reproducibility for LF, LFpen and FC in both groups.

Condition	ICC (95% Confidence Interval) Group A		
	LF	LFpen	FC
I	0.90 (0.82 to 0.95)	0.87 (0.73 to 0.93)	0.85 (0.75 to 0.91)
II	0.87 (0.77 to 0.93)	0.91 (0.79 to 0.95)	0.86 (0.77 to 0.91)
III	0.86 (0.77 to 0.92)	0.88 (0.76 to 0.93)	0.92 (0.87 to 0.96)
Condition	ICC (95% Confidence Interval) Group B		
	LF	LFpen	FC
I	0.85 (0.72 to 0.92)	0.83 (0.72 to 0.90)	0.89 (0.77 to 0.94)
II	0.83 (0.65 to 0.91)	0.78 (0.63 to 0.87)	0.94 (0.89 to 0.96)
III	0.94 (0.89 to 0.97)	0.90 (0.84 to 0.94)	0.86 (0.77 to 0.92)



CAPÍTULO 2

Title: Validity of conventional and fluorescence-based methods for occlusal caries detection: an in vivo study with histological validation

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Short title: Methods for occlusal caries detection in vivo

Key words: Occlusal Caries, Caries Detection, Visual Examination, Radiography, Fluorescence, Clinical Study, Permanent Teeth

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Declaration of interests

The undersigned authors declare that they have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that might introduce bias or affect their judgment or that could be construed as influencing the position presented herein or the review of the manuscript entitled "*Validity of conventional and fluorescence-based methods for occlusal caries detection: an in vivo study with histological validation*".

Abstract

The aims of this in vivo study were to (1) propose clinical cut-off limits for DIAGNOdent 2095 (LF), DIAGNOdent 2190 (LFpen) and VistaProof (FC) and (2) to evaluate the validity of conventional and fluorescence-based methods for occlusal caries detection in permanent teeth. A total of 105 posterior permanent teeth with an indication for extraction were selected in 88 adult patients. After prophylaxis, one to four occlusal sites per tooth varying from sound to different stages of caries were selected. One experienced examiner assessed the teeth using LF, LFpen, FC, ICDAS and bitewing radiographs (BW). After extraction, one site was randomly chosen and assessed by histological analysis. Optimal clinical cut-off limits were proposed for LF, LFpen and FC. All methods were compared by means of sensitivity, specificity, accuracy and area under the ROC curve (A_z). The specificities/sensitivities at D_1 and D_3 were 0.60/0.93 and 0.77/0.52 (ICDAS), 1.00/0.29 and 0.97/0.44 (BW), 1.00/0.85 and 0.77/0.81 (LF), 0.80/0.89 and 0.71/0.85 (LFpen) and 0.80/0.74 and 0.49/0.85 (FC), respectively. The accuracy values were higher for ICDAS, LF and LFpen at D_1 and D_3 thresholds compared to BW and FC. The A_z varied from 0.64 to 0.95, confirming the good validity of the methods. It can be concluded that ICDAS, LF and LFpen demonstrated good validity in detecting occlusal caries in vivo. However, BW and FC showed the lowest validity to detect lesions at the D_1

and D_3 thresholds, respectively. The fluorescence-based methods cut-off limits should not be considered as exact thresholds for dentists' treatment decision.

Introduction

Although there has been a general decline in the prevalence of dental caries in many industrialised countries in the last decades, the proportion of occlusal caries has increased and its detection has become even more complicated [Mejàre et al., 1998]. This fact is explained by the widespread use of fluoride, which has increased the occurrence of non-cavitated caries lesions, resulting in advanced dentinal lesions progression beneath intact surfaces [Marthaler, 2004]. Due to the optimal fluoride levels, remineralisation occurs at the entrance of the fissure with a more resistant fluoridated hydroxyapatite layer, making demineralisation in the depth of the fissure possible.

Clinical caries detection is commonly based on visual inspection and subjective assessments of colour, translucence and dental hardness [Ekstrand et al., 1997; Nyvad et al., 1999] and on radiographic examination. However, these methods exhibit high specificity and low sensitivity for occlusal caries detection [Bader and Shugars, 2004]. More recently, the International Caries Detection & Assessment System (ICDAS) was developed and proposed to reduce the visual inspection limitation by providing six distinct severity stages of caries lesions from initial changes visible in the enamel to frank cavitation in the dentine [Ferreira Zandoná and Zero, 2006; Ekstrand et al., 2007; Ismail et al., 2007]. The ICDAS assessment is based

on the visual inspection of clean and plaque-free surfaces in both wet and dried conditions. Some studies have shown good performance of ICDAS for in vitro occlusal caries detection in permanent teeth [Ekstrand et al., 2007; Jablonski-Momeni et al., 2008; Rodrigues et al., 2008; Diniz et al., 2009; Zandona et al., 2009] and good feasibility in epidemiological studies [Kühnisch et al., 2008; Braga et al., 2009].

Because the detection of dental caries is not an easy task, new methods have been proposed to improve caries detection and to quantify early lesions on smooth and occlusal surfaces. Some of these methods are based on the fluorescence phenomenon because there is a difference in fluorescence observed between sound teeth and demineralised dental tissues [Hibst et al., 2001; Bader and Sugars, 2004]. It has been shown that bacteria emit fluorescence because they contain porphyrins, molecules that are excited by a light source with a specific excitation wavelength [Hibst et al., 2001].

In 1998, the first laser fluorescence device (LF; DIAGNOdent 2095, KaVo, Biberach, Germany) was introduced as an adjunct for dental caries detection. This device is able to capture, analyse and quantify the fluorescence emitted from bacterial porphyrins and other chromophores when the surface is illuminated by its diode laser with a wavelength of 655 nm [Hibst et al., 2001]. The amount of fluorescence intensity is seen as an

increased reading [Lussi et al., 1999; Heinrich-Weltzien et al., 2003]. In 2005, a new laser fluoresce device (LFpen; DIAGNOdent 2190, KaVo, Biberach, Germany) was developed for occlusal and approximal caries detection [Lussi and Hellwig, 2006; Lussi et al., 2006]. Both devices are based on the same principle. Some studies have reported good validity and reproducibility of the LF and the LFpen for occlusal caries detection in vitro [Lussi et al., 1999; Lussi and Hellwig, 2006; Rodrigues et al., 2008] and in vivo [Lussi et al., 2001; Heinrich-Weltzien et al., 2003; Krause et al., 2007; Huth et al., 2008; Diniz et al., 2009].

Recently, a new fluorescence camera (FC; VistaProof, Dürr Dental, Bietigheim-Bissingen, Germany) was developed for caries detection. This device emits blue light at 405 nm and captures fluorescent images from dental surfaces [Rodrigues et al., 2008]. The specific software filters and quantifies the fluorescence emitted by the tissue and converts the relationship between green and red fluorescence into numerical values, according to the pixel numbers in each image [Thoms, 2006]. Higher values indicate more severe caries lesions. However, only one study has evaluated the in vitro performance of this method for occlusal caries detection in permanent teeth [Rodrigues et al., 2008], and one study has shown high reliability in detecting caries on occlusal and smooth surfaces in primary teeth [De Benedetto et al.,

2010]. However, no clinical study has been performed to test its performance for caries detection.

The clinical performance of fluorescence-based methods is related to the cut-off limits used in clinical practice, which enable the professionals to carry out the appropriate treatment. Different cut-off limits have been suggested for the LF device, not only by the manufacturer but also by in vitro [Lussi et al., 1999; Lussi and Hellwig, 2006; Rodrigues et al., 2008] and in vivo studies [Lussi et al., 2001; Diniz et al., 2009]. For the LFpen device, some in vitro cut-off limits have been proposed [Lussi and Hellwig, 2006; Rodrigues et al., 2008], and only one study has been published regarding the clinical cut-off limits for occlusal caries detection [Huth et al., 2008]. To date, there are no clinical cut-off limits published for the FC device. Consequently, more clinical studies are required to establish the most appropriate cut-off limits for fluorescence-based methods.

Another fundamental point is related to the magnitude of the gold standard method in a clinical research study. According to Heinrich-Weltzien et al. [2003], clinical examination in teeth indicated for extraction due to orthodontic or other reasons followed by histological validation would be the approach to solve the ethical problem of validating sound and enamel carious surfaces. To date, one published study has been performed to evaluate the effectiveness of the LF device to detect occlusal caries in permanent teeth

under clinical conditions where histological validation was used as a gold standard after tooth extraction [Angnes et al., 2005].

Therefore, the aims of this in vivo study were to (1) propose clinical cut-off limits for the LF, the LFpen and the FC devices and (2) to evaluate the clinical validity of conventional and fluorescence-based methods for occlusal caries detection in permanent teeth using the histological gold standard for total validation of the sample.

Materials and Methods

This study was conducted in accordance with the declaration of Helsinki, and it was approved by the ethics committee in research of the Araraquara Dental School (04/08). The study aim, procedures, safety and benefits were explained to the subjects. Signed and informed consent was obtained from all volunteer subjects before the start of the examination.

Participant Selection

The participants were recruited from patients aged between 18 and 35 years attending the Department of Oral and Maxillofacial Surgery of the School of Dentistry of Araraquara. Eighty-eight patients who had at least one posterior tooth indicated for extraction due to periodontal disease or

orthodontic reasons were involved in this in vivo study. Patients with severe diseases or syndromes were not included.

A total of 105 posterior permanent (40 premolars and 65 molars) with macroscopically intact occlusal surfaces or presenting with different stages of caries lesions were selected. Teeth with caries lesions of the approximal, buccal or lingual surfaces, frank occlusal cavitation, fillings, pit and fissure sealants, hypoplasia, orthodontic bands and third molars in eruption were not included.

The occlusal surfaces were cleaned carefully with a rotating brush and water using a low-speed handpiece [Anttonen et al., 2005]. An independently trained examiner who was not involved in the clinical examinations was shown one to four easily re-located occlusal sites of each tooth as potential investigation sites (190 total sites) [Jablonski-Momeni et al., 2008]. These sites were recorded and identified on a simple drawing of the occlusal surface to guide lesion location. Digital images of the occlusal surfaces were taken with a digital camera (EOS XSi; Cannon, Tokyo, Japan) with a 100-mm lens.

The clinical examinations were carried out before the tooth extraction by one experienced dentist with previous experience in caries detection methods. No intra-examiner reproducibility was performed because the teeth were examined on the same day that they were scheduled to be extracted. A

chair side assistant handled all information collection, which was not available to the examiner.

Visual Examination

Visual examination was performed with patients positioned in a dental chair and with the aid of a light reflector, air/water spray, and dental mirror (size 5) using the ICDAS criteria, ranging from sound surfaces to extensive cavitation. First, they were analysed moist and then dried. A ball-ended explorer was used without applying pressure to confirm only cavitation. The examiner had previous experience using the ICDAS criteria.

The caries status was recorded as suggested by Jablonski-Momeni et al. [2008] as follows: (0) sound tooth surface: no evidence of caries after prolonged air drying (5 s); (1) first visual change in enamel: opacity or discoloration (white or brown) is visible at the entrance to the pit or fissure after prolonged air drying, which is not or hardly seen on a wet surface; (2) distinct visual change in the enamel: opacity or discoloration distinctly visible at the entrance to the pit and fissure when wet, lesion must still be visible when dry; (3) localised enamel breakdown due to caries with no visible dentin or underlying shadow: opacity or discoloration wider than the natural fissure /fossa when wet and after prolonged air drying; (4) underlying dark shadow from dentin ± localised enamel breakdown; (5) distinct cavity with visible

dentin: visual evidence of demineralisation and dentin exposed; and (6) extensive distinct cavity with visible dentin and more than half of the surface involved.

Radiographic Examination

Bitewing radiographs were taken at the beginning of this study, following the principles of dental treatment planning. They were examined using an X-ray viewer and an X-ray film magnifier (magnification 2x; VRX - Fabinject, Taubaté - São Paulo, Brazil) in a dark room to determine the presence or absence of radiolucency. Caries was recorded as follows: (0) no radiolucency, (1) radiolucency in the enamel, (2) radiolucency in the outer half of the dentin, and (3) radiolucency in the inner half of the dentin [Rodrigues et al., 2008].

LF and LFpen Assessments

After visual examination, each occlusal surface was examined using a DIAGNOdent 2095 (LF; KaVo, Biberach, Germany) and a DIAGNOdent 2190 (LFpen; KaVo, Biberach, Germany). The LF and LFpen measurements were performed using a fibre-optic conical tip (tip A), specifically designed for occlusal surfaces, and the cylindrical sapphire fibre tip, respectively, and according to the manufacturer's instructions.

Before each measurement, the devices were calibrated with a ceramic standard, and the fluorescence of a sound spot on the cuspal area of the buccal surface was recorded for each tooth (zero value) to provide a baseline value for that tooth. Sites were assessed under cotton roll isolation and after briefly being air-dried with a 3-in-1 syringe. The tip was positioned perpendicular to the test sites and rotated around its long axis, according to the manufacturer's instructions. The highest reading shown on the display was recorded, and the zero value was subtracted from this value [Rodrigues et al., 2008].

FC Assessments

The FC measurements were performed under cotton roll isolation and after briefly being air-dried with a 3-in-1 syringe in a dark environment. The type of spacer tip was selected according to the patient's mouth opening. The thinner or the thicker spacers provide distances of around 0.5 cm and 1.0 cm, respectively, between the tip and the tooth [De Benedetto et al., 2010]. After capturing the images, they were analysed by the FC-specific software (DBSWIN, Dürr Dental, Bietigheim-Bissingen, Germany), which translates the red and green rate of fluorescence to numbers that correspond to the lesion severity [Rodrigues et al., 2008]. The values were recorded for further analysis.

Tooth Extraction

All teeth were extracted very carefully under local anaesthesia by means of forceps/elevators techniques by residents in oral and maxillofacial surgery under strict asepsis. After extraction, each tooth was immediately frozen at - 20°C [Francescut et al., 2006].

Validation

For validation, one site was randomly chosen for each tooth with more than one investigation site and was used as the “test site” for that tooth because multiple investigation sites on a single tooth do not represent statistically independent data [Jablonski-Momeni et al., 2008]. Black and white copies of the digital photographs, printed on draft quality paper, were used for test site localisation.

First, the teeth were defrosted for 3 hours, the calculus and debris were removed using a scaler and then they were cleaned for 15 s with water and a toothbrush. After that, they were hemi-sectioned in a buccal to lingual direction near to the centre of the test site using a water-cooled diamond blade of 0.5 mm in thickness (ISOMET 1000, Buehler Ltda., Lake Bluff, II, USA). Then they were ground using silicon carbide paper with decreasing grain size of 400, 600, 1200 and 2000. The progression of the grinding process was constantly checked under a stereomicroscope (SZX7, Olympus,

Tokyo, Japan) with a magnification of 10x until the periphery of the site was reached. The teeth surfaces were then coloured with saturated rhodamine B (Fluka, Buch, Switzerland), and histological examination was performed according to the rhodamine B penetration into either the enamel or into both the enamel and dentine tissues. The sites were assessed for caries extension (magnification 10x) according to Lussi et al. [1999] as follows: (0) caries free, (1) caries extending up to halfway through the enamel, (2) caries extending into the inner half of enamel, (3) caries in the dentine, and (4) deep dentine caries.

Statistical Analysis

The data were analysed using the statistical software MedCalc (version 9.3.0.0, Mariakerke, Belgium), and the level of significance was $p<0.05$. The optimal cut-off limits for LF, LFpen and FC were determined by the highest sum of sensitivity and specificity at each threshold. Sensitivity, specificity, accuracy and area under the ROC curve (A_z) were calculated at the D_1 (considering as disease gold-standard scores from 1 to 4) and D_3 (considering as disease both gold-standard scores 3 and 4) diagnostic thresholds. The McNemar test ($p<0.05$) was applied to compare the validity among the methods. In addition, a nonparametric statistical test was applied to assess the difference of the areas under the ROC curve (A_z) [Hanley and

McNeil, 1983]. Spearman's rank correlation coefficient was used to evaluate the agreement of the different methods with histology scores.

Results

The current study included a total of 105 permanent teeth (42 maxillary molars, 23 mandibular molars, 24 maxillary premolars and 16 mandibular premolars). From the 105 occlusal test sites assessed, histological examination showed that 5 (4.75%) were classified as caries free, 14 (13.5%) as having caries extending up to halfway through the enamel, 59 (56%) as having caries extending into the inner half of the enamel, 16 (15.25%) as having caries in the dentine, and 11 (10.5%) as having deep dentine caries.

The optimal cut-off limits for the LF, LFpen and FC devices were determined from the ROC curve in such a way that the best performance was achieved, as shown in Table 1.

Table 2 presents the specificity, sensitivity, accuracy and area under the ROC curve (A_z) for ICDAS, BW, LF, LFpen and FC at the D_1 and D_3 thresholds. At the D_1 threshold, the highest specificities were observed for BW (1.00) and LF (1.00), although no statistically significant difference was observed among the values for all methods. The highest sensitivity and accuracy values were shown by ICDAS (0.93 and 0.91), LF (0.85 and 0.86) and LFpen (0.89 and 0.89). However, BW demonstrated statistically lower

sensitivity (0.29) and accuracy (0.32). At the D_3 threshold, the highest specificity was shown for BW (0.97) and the lowest for FC (0.49). On the other hand, the highest sensitivities values were observed for LF (0.81), LFpen (0.85) and FC (0.85), with no statistically significant difference among them. The highest accuracy value was observed for BW (0.84) and the lowest for FC (0.58), whereas ICDAS, LF and LFpen showed similar values. The A_z values at the D_1 and D_3 thresholds varied from 0.64 (BW) to 0.95 (LFpen) and from 0.72 (FC) to 0.84 (LF), respectively.

Figure 1 represents the comparison of the ROC curves for all methods at the D_1 and D_3 thresholds, and it differentiates the performance among the methods. At the D_1 threshold, ICDAS, LF and LFpen showed the highest areas under the ROC curve, while LF and LFpen presented the highest areas at the D_3 threshold.

Spearman's correlation coefficients are shown in Table 3. The values varied from 0.35 (FC) to 0.55 (LFpen), indicating poor to moderate agreement of the different methods with histology scores.

Discussion

Occlusal surfaces are the most commonly affected site for caries lesions and represent a diagnostic dilemma for clinicians. The presence of stains, complex anatomy, and the limitations related to conventional methods

ensure that any device that improves the detection of occlusal caries would be well received to provide diagnostic assistance. In this study, the validity of fluorescence-based methods was compared with visual and radiographic examination for occlusal caries detection under clinical conditions.

It is known that sound teeth and enamel caries in occlusal surfaces cannot be validated under clinical conditions for ethical reasons [Heinrich-Weltzien et al., 2003]. According to these authors, clinical examination of sound teeth destined for extraction for orthodontic or other reasons followed by histological validation in the laboratory would be the approach to solve this problem. To date, there is only one published study of cut-off limits histologically validated for the LF device of sound occlusal surfaces in permanent teeth under clinical conditions [Angnes et al., 2005]. In their study, a total of 110 sites were selected from 57 third molars indicated for extraction. Many in vivo studies are limited to premolars and/or third molars, where extraction can be scheduled. The problem is that first and second molars are the teeth most frequently experiencing occlusal caries due to their pronounced pit and fissures system, which, in ways, may affect the performance of diagnostic methods [Bader et al., 2002]. In our study, apart from the above limitations, the sample consisted of 105 posterior teeth (38% premolars and 62% molars, being 95% third molars) scheduled for extraction. It must be remembered that most of the occlusal surfaces of third molars tend

to have more fissures**,** which are often less well coalesced. Therefore, the prevalence of caries in third molars erupted in patients 25 years of age or older is high and not unique because they also have caries experience in first and second molars [Shugars et al., 2004].

According to Bader et al. [2002], based on a systematic review of the literature, the choice of sites rather than surfaces may cause a risk to external validity; this is because most occlusal surfaces will present with multiple sites for assessment because the results of site assessment do not summarise the status of the entire surface, as is routinely done in clinical practice. In view of this fact, in our study to allow for the assessment of the entire occlusal surface, one to four sites on each tooth were chosen as potential investigation sites. Because the data collected from multiple sites cannot be treated as independent [Jablonski-Momeni et al., 2008], only one occlusal site per tooth was randomly chosen for histological validation after clinical examination, which is different from the study performed by Angnes et al. [2005].

Performance of fluorescence-based methods is dependent on the cut-off limits used. Because different cut-off limits have been proposed and evaluated, it is not surprising that the great variation in the results is confusing to clinicians who are seeking answers from the literature. It is important to point out that cut-off limits obtained in clinical settings are different from those

in laboratory settings. So, in vitro cut-off limits should not be transferred to the clinical situation because some factors might influence the results, such as the storage method of the extracted teeth [Francescut et al., 2006]. Besides, the difference in cut-off values for sound teeth, enamel or dentine caries will affect the treatment decision-making in clinical practice [Heinrich-Weltzien et al., 2003]. Some studies have proposed guidelines for the clinical use of laser fluorescence values. Thus, it is important to stress that the values obtained with the LF and LFpen are not exactly the same. The fluorescence values obtained using the LFpen in vivo were significantly lower than those obtained using the LF device [Krause et al., 2007]. In contrast, the data from the present study showed that the fluorescence values obtained by the LFpen device were higher than those from the LF. Others studies have also showed higher fluorescence values for the LFpen device [Diniz et al., 2008; Rodrigues et al., 2008]. The reasons for this finding could be due to the different diameters and materials of the tips in both devices, which might influence the extent of the excitation light and the capture of the fluorescence emitted by the dental tissues.

In our study, the optimal cut-off limits of the occlusal surfaces were obtained by determining the sum of specificity and sensitivity values. Based on the results, the following cut-off limits for the clinical use of the LF device were described: values of 0-4 = no caries, values of 5-27 = enamel caries,

and values >27 = dentine caries. However, our findings are different from those published in other clinical studies, which have shown that values from 0 to 14 represent sound surfaces and values from 14 to 29 represent enamel caries [Lussi et al., 2001; Diniz et al., 2009]. These differences could be attributed to the validation method used in those studies, where surfaces assessed as caries free were not validated by fissure opening, and the surfaces assessed as carious based on visual and radiograph examination were validated by operative intervention. In addition, the small number of sound teeth (4.75%) and the great number of teeth with enamel caries (69.5%) in our sample may have influenced the results. In our study, histological examination was used for validation due to its ability to identify minimal changes in the dental tissues. Furthermore, operative intervention can confirm a positive diagnosis but cannot detect false negatives. Thus, the borderline reading for the operative intervention found in our study should be considered at a peak value of above 27. This is a value of the same magnitude as reported earlier by Lussi et al. [2001], who observed that values superior than 29 must be considered for operative care. The difference of two units may not lead to false decisions because the LF device should only be used as a second opinion. It is noteworthy that other factors, such as patient caries risk, are also involved to the treatment decision-making, and, in no case should early caries detection be an excuse for early operative

intervention, unless this is indicated by other clinical parameters [Huth et al., 2008]. According to Lussi et al. [2001], a higher setting for operative intervention represents a safety factor for teeth with stains or calculus on the occlusal surfaces. Khalife et al. [2009] reported that values between 35 and 40 indicate dentine caries. On the other hand, Diniz et al. [2009] found that values greater than 21 indicate dentinal decay. Most likely, this divergence is related to the samples used in their study, which were permanent molars in patients aged between 7 and 12 years.

For the LFpen, the different cut-off limits proposed by the manufacturer and by an in vitro study [Lussi and Hellwig, 2006] were not clinically validated. Recently, an in vivo study published the cut-off limits for the LFpen for occlusal caries detection in permanent teeth [Huth et al., 2008] as follows: 0-12 = sound, 13-25 = enamel caries, and >25 = dentine caries; in our study, the cut-off limits were as follows: 0-4 = sound, 5-32 = enamel caries, and >32 = dentine caries. These difference may be due to the fact that in their study, the cases where no operative intervention was indicated the site was reassessed visually and radiographically after 12 months to check the caries status and to serve as a reference in the validation analysis. Another clinical study found that LFpen values greater than 23 indicate dentine caries [Krause et al., 2007]. Nevertheless, this study has limitations because the enamel lesions were not validated by operative intervention for ethical reasons.

The present study investigated the FC device for occlusal caries detection in vivo for the first time. Regarding the cut-off limits, our results showed that the values presented by the software analysis were different from the values proposed by the manufacturer. The software highlights the caries lesions in different colours and defines their extension on a scale from 0 to 5: 1.0-1.5 (blue) indicates beginning enamel caries, 1.5-2.0 (red) indicates deep enamel caries, 2.0-2.5 (orange) indicates dentine caries, and 2.5-5.0 (yellow) deep indicates dentine caries. According to our cut-off limits, 0-1.2 indicates sound surfaces, 1.3 indicates enamel caries, and >1.3 indicates dentine caries. These data are comparable to a recently published in vitro study which mentions that values from 0 to 1.262 represent sound surfaces, 1.263-1.299 represent caries lesions in the outer half of the enamel, 1.300-1.319 represent caries lesions in the inner half of the enamel, and >1.319 represent dentine caries [Rodrigues et al., 2008]. In that study, the teeth were frozen after extraction, and no loss of fluorescence occurred, which makes the results comparison between in vitro and in vivo conditions viable. The FC device used in their study was a prototype and showed cut-off limits so close to each other that their use in clinical practice would be difficult, if not impossible [Rodrigues et al., 2008]. Currently, this device has been modified and provides more objective values, although the limits still remain close to each other.

In general, teeth should be cleaned and dried to aid in the correct detection of caries lesions by regular visual examination prior to fluorescence measurements. Several reports have pointed out that plaque, remnants of prophylaxis pastes, powders or gels, stains and dental materials could influence the fluorescence readings and give false-positive results [Lussi et al., 1999; Anttonen et al., 2005; Lussi and Reich, 2005; Diniz et al., 2008]. Therefore, in the present study, professional cleaning of the occlusal surfaces was performed using a rubber cup and plain water spray, as recommended by Anttonen et al. [2005]. Another important concern is related to the sterilisation process of the fluorescence-based methods tips for infection control. Some studies have shown that sterilisation of the tips in autoclave influences LF readings and reduces the performance in detecting occlusal caries [Cabral et al., 2006; Rocha-Cabral et al., 2008]. Thus, in this present study, we used a PVC seal wrap on LF and LFpen tips as a barrier against cross-infection during the clinical examinations. For the FC device, we used the protective plastic coat provided by the manufacturer. This procedure for infection control has been recommended in clinical practice [Cabral et al., 2006; Rodrigues et al., 2009; De Benedetto et al., 2010].

In this clinical study, ICDAS showed high sensitivity (0.93) and moderate specificity (0.60) at the D_1 threshold, confirming its good ability to detect occlusal caries lesions in permanent teeth, especially early clinically

visible changes in enamel due to carious demineralisation. This result has also been shown by laboratory studies [Ekstrand et al., 2007; Jablonski-Momeni et al., 2008; Diniz et al., 2009; Zandona et al., 2009]. ICDAS criteria also showed high accuracy (0.91) and high area under the ROC curve (0.86). For the visual inspection using ICDAS criteria, first the surfaces of the teeth were analysed under wet conditions and then after careful drying with pressurised air, which clearly visualises the opaque appearance of demineralisation. At the D_3 threshold, ICDAS demonstrated moderate sensitivity (0.52) and good specificity (0.77). Jablonski-Momeni et al. [2008], in an in vitro study, also reported a higher specificity than sensitivity for the detection of dentine caries. In contrast, Rodrigues et al. [2008] and Diniz et al. [2009] reported good sensitivity (0.73 and 0.75, respectively) for in vitro dentine caries detection. These differences could be explained by the clinical condition for the visual examination and the small number of dentine lesions found in our study, which probably reflects the greater difficulty to distinguish hidden caries in permanent teeth. Besides, in this study, the correlation between ICDAS criteria and the histology scores was not strong (0.48). Jablonski-Momeni et al. [2008] and Diniz et al. [2009] also found a fair correlation. However, in a previous study, this correlation was stronger (0.73) [Zandona et al., 2009]. It should be emphasised that there is no current data available on the clinical validity of ICDAS for occlusal caries detection.

Concerning the radiographic examination at the D_1 and D_3 thresholds, BW achieved the highest specificities (1.00 and 0.97, respectively) and the lowest sensitivities (0.29 and 0.44, respectively). In agreement with our study, Angnes et al. [2005] and Chu et al. [2010] also found lower sensitivity and higher specificity values for BW to detect dentine caries and enamel caries on the occlusal surfaces of permanent teeth in vivo. This similarity is related to the low dentine caries prevalence, which has a profound effect on the ability of the BW to correctly detect those lesions [Angnes et al., 2005]. However, Lussi et al. [2001] reported moderate sensitivity (0.63) and high specificity (0.99) at the D_3 threshold. In the present study, because the radiographs were taken for reasons other than our study, it can be suggested that the radiographs images may have varied, thereby affecting the BW validity. Anttonen et al. [2003] suggested that radiographic examination is the least accurate method not only for enamel caries detection but also for dentine caries. In our study, BW demonstrated the lowest accuracy (0.32) for enamel caries detection and the highest accuracy (0.84) for dentine caries detection. Spearman's correlation coefficient for BW was moderate (0.48) and equal to that of ICDAS. On the other hand, Alkurt et al. [2008] described a lower correlation for BW and for visual examination (0.22). In practice, bitewing radiography, especially for occlusal caries confined to the enamel, must be used as an adjunct to the visual examination.

Bader and Shugars [2004] found that LF demonstrated superior sensitivity but inferior specificity to visual examination. In contrast, we observed that LF showed good specificity and accuracy. In the present study, LF showed a sensitivity of 0.85 and a specificity of 1.00 for enamel caries detection. Comparing the results obtained in this study with those of other authors, we observed that the sensitivity level of 0.85 obtained is higher than the 0.40 obtained by Barbería et al. [2008]. This discrepancy could be due to the kind of sample used in their study, with a limited percentage of enamel caries (30%) and a high percentage of sound teeth (64%). Concerning dentine caries detection, LF showed high sensitivity and specificity values (0.81 and 0.77, respectively). Similar results were also described in other clinical studies [Abalos et al., 2009; Chu et al., 2010]. However, Lussi et al. [2001] and Diniz et al. [2009] found higher specificity values (0.86-0.87), while other studies showed lower specificity (0.54-0.56) [Angnes et al., 2005; Krause et al., 2007]. The correlation of LF measurements with the histology scores was better than ICDAS and BW. This finding was also reported by Alkurt et al. [2008]. Several studies recommend using the LF device as a useful adjunct in the decision-making process in cases of doubt after visual examination [Lussi et al., 2001; Heinrich-Weltzien et al., 2003; Krause et al., 2007; Diniz et al., 2009]. However, in an epidemiological study, the laser

fluorescence device seems to bring no additional detection gain when ICDAS criterion is used [Kühnisch et al., 2008].

The present study showed that the LFpen presented similar performance when compared to LF, with no statistically significant difference between them. As both devices are based on the same physical principle, it is likely that their similar performance could also be expected. At the D_1 and D_3 thresholds, the LFpen demonstrated high sensitivity (0.89 and 0.85, respectively) and specificity (0.80 and 0.71, respectively) values. High specificity values were also reported by Huth et al. [2008]. However, other studies showed lower sensitivity values for dentine caries detection in vivo [Krause et al., 2007; Huth et al., 2008]. This may be due to the different cut-off limits used for the performance calculation. The LFpen also demonstrated high accuracy, especially for enamel caries detection. These results are consistent with the results reported by Huth et al. [2008] in an in vivo study. Although the LFpen has shown the highest correlation with histology scores (0.55), it can be considered a moderate correlation. Unfortunately, there are few in vitro studies and two studies investigating the clinical performance of the LFpen for occlusal caries detection, which makes the comparison more restricted. In addition, the extrapolation of the in vitro results for the clinical practice is inappropriate.

Regarding the new fluorescence camera, the FC device showed good performance, especially for detecting caries lesions. The sensitivity values were 0.74 and 0.85 at the D_1 and D_3 thresholds, respectively. However, the sensitivity for enamel caries (0.74) and the specificity for dentine caries (0.49) were statistically lower when compared between the LF and LFpen devices. For dentine caries, the sensitivity was similar between them. These results could be expected, considering that the FC device detects increases in fluorescence using the same principle as the LF and the LFpen devices [Thoms, 2006; Rodrigues et al., 2008]. In the literature, only one study has been performed in vitro to evaluate its performance in detecting occlusal caries lesions in permanent teeth [Rodrigues et al., 2008]. Another in vitro study has evaluated the reliability of the method and showed a higher reliability for occlusal and smooth surfaces, similar to the LF and LFpen [De Benedetto et al., 2010]. In agreement with Rodrigues et al. [2008], our study also showed a weak correlation for the FC device with the histology scores. In our study, the type of the spacer tip for the FC device was selected according to the patient's mouth opening. According to De Benedetto et al. [2010], there are no differences between FC measurements obtained using thinner or thicker spacers. The FC's manufacturer advises the use of the distance spacers placed in the camera to reach a reproducible location, to keep the

best distance to the tooth surface and to achieve the optimum image quality without further focusing [De Benedetto et al., 2010].

As this is the first study evaluating the clinical validity of the new fluorescence camera (FC), it is important to discuss its advantages and limitations to provide clinicians with a better understanding of this method. In FC images, large areas of the dental surfaces can be inspected within seconds, which is not possible for the LF or LFpen, which use only a fibre-optic probe to check fluorescence intensities at a single point on the tooth surface. Compared to in vitro experimental conditions, in vivo application of FC has certain difficulties, including access to lesions on occlusal surfaces located more posterior in patients with limited mouth opening, moisture in the oral environment and angulations of the light source. It could be suggested that some factors may influence the quality of the FC image recording caries status, such as the presence of plaque, calculus and/or staining on the tooth surface, and the complex light scattering pattern generated on the occlusal surfaces, which can make the reconstruction of reliable fluorescence values difficult. It must be pointed out that ambient light, daylight or office light may also influence the quality of the FC images. Thus, images should be captured under partial blackout conditions.

The area under the ROC curves (A_z) demonstrated that the LF and LFpen followed by ICDAS were the most accurate methods for occlusal

caries detection. At the D_1 threshold, the LF and LFpen demonstrated the highest A_z values when compared to the other methods. In contrast, at the D_3 threshold, statistically significant differences were found between the LF and FC A_z values. Some in vivo studies have also shown higher A_z values for the LF [Heinrich-Weltzien et al., 2003; Abalos et al., 2009; Diniz et al., 2009] and for the LFpen [Huth et al., 2008]. An advantage of ROC analysis is that it reflects the diagnostic validity more comprehensively than sensitivity and specificity, providing an overall validity of the methods.

In the present investigation, the intra- and interexaminer reproducibility of the conventional and florescence-based methods were not evaluated because of the short time available to perform the clinical examinations before tooth extraction. However, it is essential that the caries detection methods demonstrate good reproducibility so that reliable results can be obtained at different assessments. In addition, intra- and interexaminer agreement is considered the first item in evaluating detection methods [Kühnisch et al., 2007].

In view of the findings in this in vivo study, within its limitations, it is reasonable to conclude that the clinical cut-off limits proposed by the fluorescence-based methods (LF, LFpen and FC) revealed a considerable outcome in validity, and they seem to be suitable for occlusal caries detection in permanent teeth. However, they should not be considered as exact

threshold measurements. In fact, the values should be used as a second opinion for dentists' treatment decisions. A crucial point is that the decision to interfere or not is dependent upon a range of other factors, such as the patient's caries risk, fluoride availability, sugar intake, and caries activity. It can be suggested that ICDAS offers promise for occlusal caries detection in clinical practice. Although BW is still the most widely used adjunct to caries detection and to clinical decision making, especially in approximal surfaces, its use should be carefully considered for occlusal caries detection. To date, clinical studies and the number of validated caries lesions are limited. Therefore, further clinical studies should be performed to evaluate the validity and the reproducibility of fluorescence-based methods for caries detection, especially the LFpen and the FC devices, and of the ICDAS visual criteria for both detection and caries activity assessments.

Acknowledgments

The authors are grateful to the Department of Oral and Maxillofacial Surgery of the School of Dentistry of Araraquara, especially Isabela Cristine Manzolli Rodrigues and the residents for their clinical assistance. The authors wish to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for their financial support. This study was submitted to

the School of Dentistry of Araraquara (Univ. Estadual Paulista – UNESP, Brazil) as a requirement for the PhD degree in paediatric dentistry.

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Table 1: Optimal cut-off limits for the LF, LFpen and FC.

Histology	Clinical lesion depth	LF	LFpen	FC
0	Sound	0-4	0-4	0-1.2
1,2	Enamel caries lesions	5-27	5-32	1.3
3,4	Dentine caries lesions	>27	>32	>1.3

Table 2: Specificity, sensitivity, accuracy and area under the ROC curve (A_z) for ICDAS, BW, LF, LFpen and FC for the clinical detection of occlusal caries at the D_1 and D_3 thresholds.

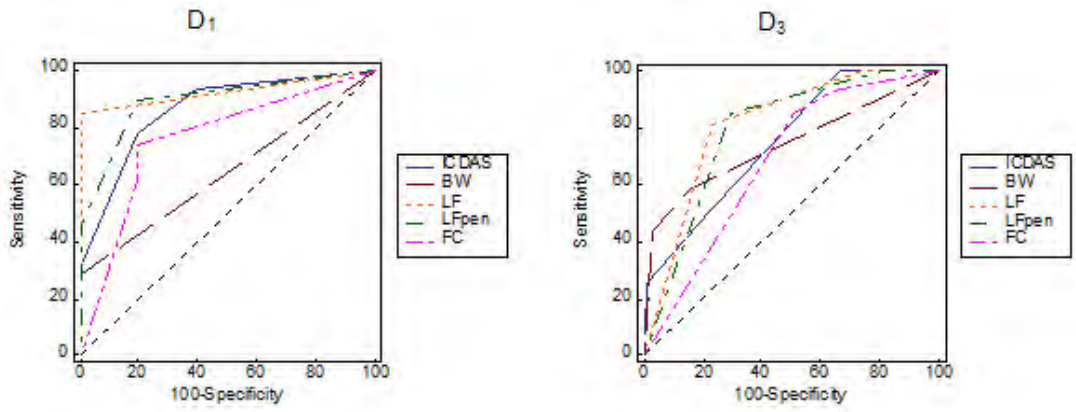
Method	D_1			
	Sp	Se	Ac	Az
ICDAS	0.60[a]	0.93[a]	0.91[a]	0.86[a]
BW	1.00[a]	0.29[b]	0.32[b]	0.64[b]
LF	1.00[a]	0.85[c]	0.86[a]	0.94[c]
LFpen	0.80[a]	0.89[a,c]	0.89[a]	0.95[c]
FC	0.80[a]	0.74[d]	0.74[c]	0.79[a]
Method	D_3			
	Sp	Se	Ac	Az
ICDAS	0.77[a]	0.52[a]	0.70[a]	0.75[a,b]
BW	0.97[b]	0.44[a]	0.84[b]	0.74[a,b]
LF	0.77[a]	0.81[b]	0.78[a]	0.84[a]
LFpen	0.71[a]	0.85[b]	0.74[a]	0.79[a,b]
FC	0.49[c]	0.85[b]	0.58[c]	0.72[b]

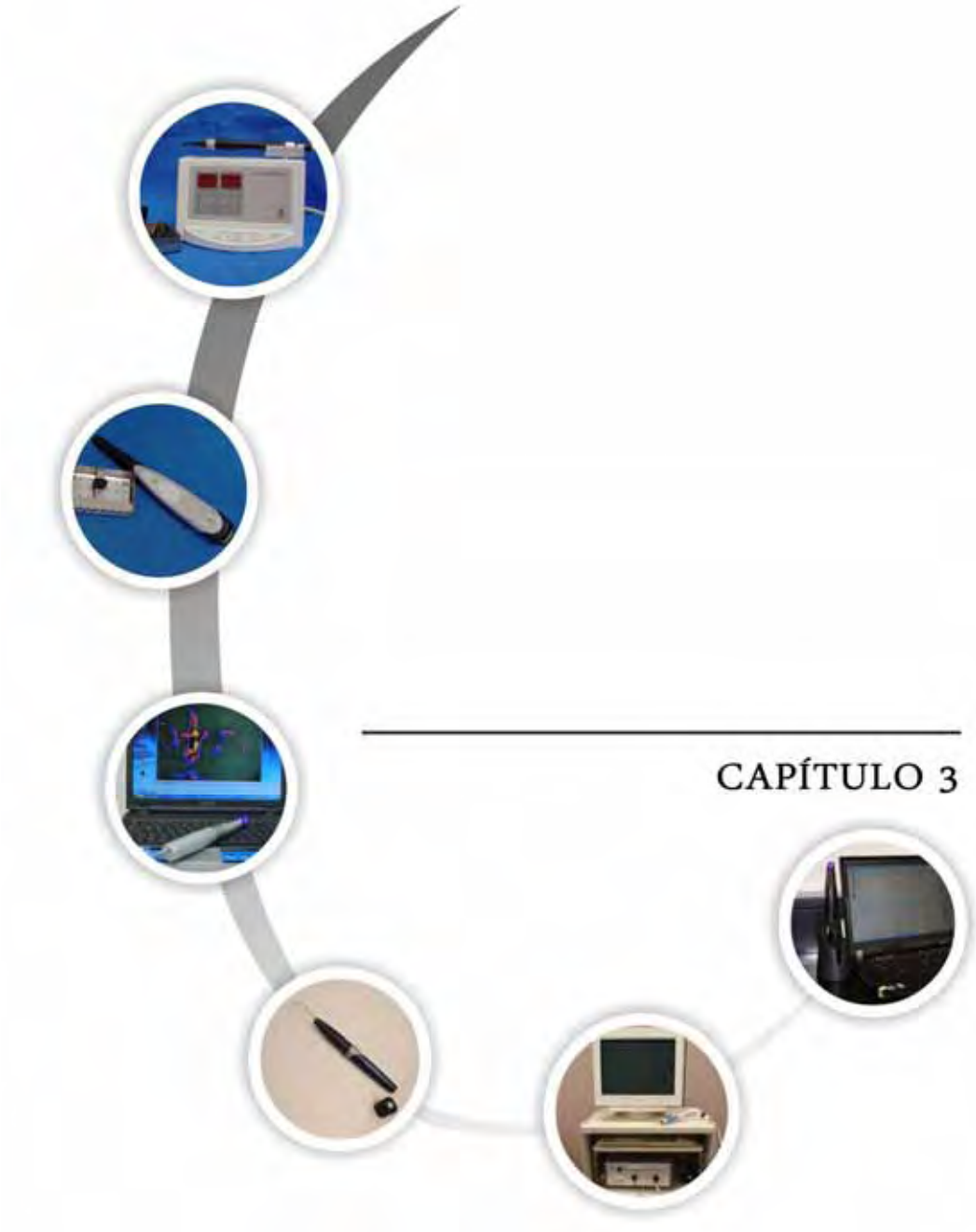
D_1: 0= sound; 1-4=decayed
D_3: 0-2=sound; 3-4=decayed
Significant differences are represented by different superscript letters, considering the same column.
(McNemar test, $p<0.05$ for specificity, sensitivity and accuracy; nonparametric statistical test for A_z)

Table 3: Spearman's correlation coefficients for the detection of occlusal caries of the different methods with histological assessment.

Method	Spearman's Coefficient
ICDAS	0.48
BW	0.48
LF	0.52
LFpen	0.55
FC	0.35

Figure 1: Comparison of ROC curves among all methods at the D_1 and D_3 thresholds.





CAPÍTULO 3

Evaluation of Occlusal Caries Activity Using Two Clinical Assessment Systems and a Novel Luminescence Assay in Primary Teeth

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Short title: Caries Activity Assessment on Occlusal Surfaces

Key-words: Occlusal caries, Visual tactile assessment, Caries activity, Primary teeth

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ABSTRACT

The aims of this study were (1) to compare the ICDAS-LAA and the clinical visual/tactile criteria for caries activity assessment in primary teeth in vivo, (2) to compare these in vivo results with the in vitro results obtained by a novel luminescence assay, and (3) to compare both criteria with the luminescence assay to determine the activity status on lesions subject to a 2-month in vivo follow-up. The two in vivo criteria were compared in 88 primary molars of 58 children aged 9-12 years. A subsample (10) was re-examined after 2 months. After exfoliation or extraction, the teeth were evaluated by a novel luminescence assay. A strong correlation between both clinical criteria was observed. Using the clinical criteria as the reference standard the luminescence assay correctly scored 7 of the 10 lesions monitored longitudinally in vivo. In conclusion, the clinical criteria exhibited a high correlation when applied in vivo. The luminescence assay showed a potential ability to assess caries activity status in vitro.

INTRODUCTION

The slow progression of dental caries has focused attention on the need for accurate methods for early caries lesion detection and strategies to control, arrest or reverse the disease process. In view of the fact that dental caries is a highly dynamic fluctuating process, involving demineralization and remineralization over a period of time (Fejerskov, 1997), the determination of the caries activity status plays a major role in the treatment decision-making.

The assessment of caries activity comprises the evaluation of etiological factors associated with the clinical examination of caries lesions (Angmar-Månsson et al., 1998). Usually, the clinical visual/tactile assessment of caries activity is based on the evaluation of clinical parameters, such as visual appearance, plaque accumulation and tactile feeling when a probe is gently run across the lesion surface. However, the criteria are based on subjective evaluation and the examiner's opinion is variable and unreliable using visual and tactile investigation to differentiate between active and inactive lesions (Ekstrand et al., 2005).

Recently, the ICDAS (International Caries Detection and Assessment System) was developed with the mission to devise a set of international visual criteria for caries detection that would also allow assessment of caries activity (Ekstrand et al., 2007). The Lesion Activity Assessment (LAA) criteria have been developed for use in association with the ICDAS scoring system based

on using weighted numerical values for lesion appearance (ICDAS score of the lesion), lesion location in relation to a cariogenic plaque stagnation area and surface integrity by tactile sensation when a ball-ended probe is gently drawn across the surface (Ekstrand et al., 2007; Varma et al., 2008). This evaluation involves the characterization of the caries lesion activity during a single clinical examination, in real time, in order to determine whether intervention is necessary (Ekstrand et al., 2009). The association of the LAA and the ICDAS codes involves lesion detection and coding, thereby estimating its depth or severity, and assessing its activity (Braga et al., 2009). An in vitro study found that there is no major difference between the Nyvad system and the ICDAS-LAA in assessing caries activity in primary teeth (Braga et al., 2009). However, in a clinical study ICDAS-LAA seems to overestimate the caries activity assessment of cavitated occlusal lesions in primary teeth compared to the Nyvad system (Braga et al., 2010).

The concept of caries activity allows classification into active/progressing or inactive/arrested lesions. Based on that, a novel assay using a luminescence marker was recently developed. The Carivis™ system (LUX DS, Lux Innovate Ltd, Edinburgh, UK) relies on the single properties of the Glowdent™ marker (LUX DS, Lux Innovate Ltd, Edinburgh, UK – patent pending) that captures the dissolved tooth minerals and produces a light signal. This signal is captured by a custom-built intra-oral imaging device. The

amount of light emitted is proportional to the amount of mineral released and the resulting image is a “demineralization map” of the tooth (Longbottom et al., 2008). Some preliminary studies (not yet published in full) have demonstrated good results in assessing caries activity status in permanent and primary teeth (Longbottom et al., 2008; Haughey et al., 2009; Perfect et al., 2009). Although these prior reports suggest a potential of this novel technology in assessing activity status, there are no scientific reports on comparing the abilities of the LAA and the clinical visual/tactile for caries activity assessment and the novel luminescence assay parameters.

Therefore, the aims of this study were (1) to compare the ICDAS-LAA and the clinical visual/tactile for caries activity assessment under in vivo conditions, (2) to compare the in vivo results from both criteria with the in vitro results obtained by a novel luminescence assay for caries activity assessment, and (3) to compare the two criteria with the luminescence assay to determine the activity status in primary molar teeth re-examined after a 2-month of follow-up.

MATERIALS & METHODS

Ethical Approval

The research protocol was approved by the Ethics Committee in Research of the Araraquara Dental School, Brazil (Protocol 48/08). Written

informed consent was obtained from subjects' guardians of all children prior to the participation in the study after explaining the purpose, procedures, discomforts, risks, and benefits involved.

Caries Activity Assessment Systems

Two criteria were used in this study: the ICDAS-LAA (Lesion Activity Assessment) and the clinical visual/tactile criteria.

Examiner Training

One experienced dentist, with 15 years' postgraduate experience and previous experience on the ICDAS criteria, was initially trained concerning the ICDAS criteria through literature. Then, the examiner completed the online ICDAS e-learning program developed by the ICDAS Foundation, which consists of a 90 minute course regarding the application and collection of the coding system. Regarding the caries activity assessment systems, the ICDAS-LAA (Lesion Activity Assessment) criteria were discussed following previous published studies (Ekstrand et al., 2007; Braga et al., 2009) and the clinical visual/tactile criteria were discussed based on details from original papers (Ekstrand et al., 2005). The training was performed in 3 phases: (I) with practical exercises using clinical photographs to characterize the clinical aspects of the different ICDAS carious lesions stages and the activity aspects

of the lesions; (II) evaluating occlusal surfaces of 25 primary extracted molars using the ICDAS criteria, the ICDAS-LAA criteria and the clinical visual/tactile criteria; and (III) clinical examination in 5 children, which were not part of this study, in a total of 40 primary molars, using the same criteria.

Participant Selection

The subjects' targets in this study were 58 children (32 males and 26 females) between the ages of 9 and 12 years (mean age = 10.28 years ± 1.47) attending the Department of Pediatric Dentistry of the School of Dentistry of Araraquara, Brazil. The inclusion criteria was that patients had to have a periapical radiograph taken recently of at least one primary molar: a) showing advanced root resorption with physiological tooth mobility, or b) which was indicated for extraction due to orthodontic reasons. Exclusion criteria were lack of cooperation, severe general diseases or syndromes and patients who were using prescribed medications.

Before clinical examinations, the caries risk assessment was determined for each patient through a questionnaire using a modification of the CAMBRA criteria proposed by Featherstone et al. (2007), regarding previous caries experience, medical history, fluoride, sugar intake, oral hygiene, among others.

A total of 88 primary molars with macroscopically intact occlusal surfaces (ICDAS score 0) or presenting carious lesions, varying from initial changes in enamel (ICDAS score 1) to underlying dark shadow from dentin (ICDAS score 4) were selected. Teeth with frank occlusal cavitation (ICDAS scores 5 or 6), caries lesions of approximal, buccal or lingual surfaces, fillings, pit and fissure sealants, fluorosis, hypoplasia or orthodontic bands were excluded.

The occlusal surfaces were cleaned carefully with a rotating brush and water in a low-speed handpiece. Digital images of the occlusal surfaces were taken with a digital camera (EOS XSi; Cannon, Tokyo, Japan) with a 100-mm lens. The clinical examinations were carried out by one experienced dentist. All dental records were carefully handled by a chairside assistant to avoid examiner bias.

After examinations, each patient was provided with continuous and comprehensive dental care proposed according to their dental needs.

Visual Examination by the ICDAS

Visual examination was performed with children positioned in a dental unit using an operating light, a 3-in-1 syringe and a plane hand mirror. A blunt probe (CPITN) was used without applying pressure to confirm cavitation, as required by the criteria. First the teeth were analyzed moist, then air-dried for

5 seconds and re-examined. The examiner attributed the highest score, ranging from 0 to 4 (Table 1), according to the most severe condition in each occlusal surface. This area was later identified and marked with a circle on the photograph by the same examiner.

Caries Activity Assessment by the ICDAS-LAA

Caries activity assessment was performed using the ICDAS-LAA scoring system using the following clinical parameters: clinical appearance (ICDAS), plaque stagnation area and tactile sensation. A blunt probe was gently run across the lesion to assess the surface texture – rough or smooth. For each lesion under investigation, the clinical parameters were recorded with the corresponding score. Then, the total score was calculated by the chairside assistant to determine the caries activity according to Braga et al. (2009), adapted from Ekstrand et al. (2007) (Table 2). The examiner was blinded to the final score in attempt to guarantee that she would not be influenced during the following examination. A total score of 4-7 indicated an inactive lesion, while a total score >7 indicated an active lesion (Braga et al., 2009).

Caries Activity Assessment by the Clinical Visual/Tactile

Caries activity was also assessed in vivo following the criteria of presence and location of plaque stagnation, surface texture, clinical appearance, translucency and color of the dental tissues. The teeth were examined after being air dried for 5 s using a ball-ended probe to gently assess the surface texture. An active lesion was recorded as a whitish lesion, porous, opaque, rough and located in an area of plaque stagnation. On the other hand, an inactive lesion was recorded as a whitish or brownish lesion, translucent and shiny, smooth and located in a non-area of plaque stagnation.

Longitudinal Clinical Data

Of the 88 teeth scored, 10 teeth were re-examined after a 2-month follow-up period. These teeth were scheduled to be re-scored due to the presence of only minor physiological tooth mobility at the beginning of the study. The examiner carried out a subjective activity assessment using the two clinical criteria for those sites previously circled at the last recorded clinical exam before tooth exfoliation or extraction.

Luminescence Analysis

After natural exfoliation or tooth extraction, each tooth was photographed using a stereomicroscope with 10x of magnification, identified and stored individually in plastic containers with 0.1% thymol solution at 4ºC.

Each tooth was rinsed with deionised water and placed on a petridish in a dark box. The teeth were imaged first under ambient lighting (10 ms capture, 2x2 binning) and then immediately after the addition of Glowdent™ (LUX DS, Edinburgh, UK) to the tooth surface (250 µL; 60 s exposure, "luminescence images"). Images were captured with a sensitive black and white CCD camera (HX916, Starlight Express, Berkshire, UK).

Luminescence images captured after the addition of Glowdent™ were false coloured using the "Royal" colour scale and the maximum greyscale value decreased from 255 to 150 (ImageJ, version 1.42o, U.S. National Institutes of Health, Bethesda, MA, USA). As the luminescent images captured were quite dark, the greyscale value was reduced from a maximum of 255 to 150 in order to make the images brighter both on screen and when printed. Since very few of the pixels in the original images had a value greater than 150, data-loss was minimal. Therefore, a maximum greyscale value of 150 was chosen to maximize the brightness of the image while effectively not discarding any information from the image. The adjustment from the greyscale value was done manually and individually for each image.

To present merged images the “royal” luminescence image was converted to a 16 bit colour image, background luminescence was removed using the flood fill tool and the light and modified luminescence image were merged by “adding” images using the arithmetic option (all channels). Background was then converted to black using the colour replacer tool (tolerance < 3). All image manipulations to provide merged images were performed in PaintShop Pro 7 (version 7.04).

One examiner looked at each image on a monitor screen and used the colour image of the same tooth as a reference to locate the same site as the circle marked on the occlusal surface on the equivalent luminescence image. The luminescence was scored subjectively at 3 levels: (0) no luminescence present (i.e. black - no light detected) (Figure 1); (1) a dull blue luminescence, but not black (Figure 2); or (2) a bright (white-blue) luminescence signal (Figure 3). The luminescence score 0 was considered negative for caries activity, while scores 1 and 2 were considered as a positive score for caries activity when a luminescence signal was captured. According to Haughey et al. (2009), lesions identified visually as inactive appeared smoother in SEM images and did not generate a light signal from the marker.

Statistical Analysis

Statistical analysis was performed using SAS (version 9.2, SAS Institute, Inc., Cary, NC, USA). The data were expressed by descriptive and analytical statistics.

Relationships among the ICDAS-LAA, clinical visual/tactile (CVT) and luminescence assessments were evaluated using Spearman's rank correlation coefficients and Mantel-Haenszel chi-square test. The associations between the CVT, ICDAS-LAA and luminescence assessments were evaluated using two-way contingency tables and were expressed by percentage agreement.

For the activity assessment, specificity, sensitivity and accuracy were calculated for the luminescence assay using the clinical visual/tactile activity scores and the ICDAS-LAA score as the gold standard. These computations were carried out using two different cutoffs: caries active if luminescence >0 and caries activity if luminescence >1.

RESULTS

The current study included a total of 88 primary molars (28 maxillary first molars, 16 maxillary second molars, 14 mandibular first molars and 30 mandibular second molars).

Concerning the caries risk assessment it was observed that 35% of the patients were considered as at low risk, 56% as at moderate and 9% as at high caries risk.

The contingency tables are showed in Table 3. According to the clinical visual/tactile activity scores and the ICDAS-LAA activity scores, 25 (28%) of the lesions were considered inactive and 63 (72%) as active. The luminescence signals showed that 19 (22%) lesions were classified as 0, 30 (34%) as 1, and 39 (44%) as score 2. The luminescence activity showed that 19 (22%) were classified as inactive lesions and 69 (78%) as active lesions.

Table 4 shows the Spearman's rank correlations for all methods. A strong correlation between CVT and ICDAS-LAA and activity scores was observed. There was a negative correlation between CVT and luminescence assessments, and between ICDAS-LAA and luminescence assessments.

Table 5 shows the values of specificity, sensitivity and accuracy for the luminescence assessments when the ICDAS-LAA or CVT scores were used as the gold standard at D_1 (luminescence signal >0 = active lesion) and D_2 (luminescence signal of >1 = active lesion).

The longitudinal clinical data is presented in Table 6. From the 10 teeth re-examined, using the 2nd ICDAS-LAA/CVT score as the validator, 7 of the 10 luminescence scores were correct. Of the 10 lesions, 2 initially inactive lesions became active, 2 initially active lesions became arrested, 2 remained

active, and 4 remained inactive. Caries lesions arrestment (2) or activation (2) was confirmed by the luminescence signals. However, from the 4 'continuing inactive' lesions, 2 were indicated as active by the luminescence signal.

DISCUSSION

Caries detection of non-cavitated lesions has become more complicated due to the slow progression and changes in the presentation of clinical features (Mejàre et al., 1998; Hannigan et al., 2000). While dental caries is a highly dynamic process, recognition of whether lesions are active or arrested is essential for selecting the appropriate management, since it represents the sum of all risk factors and their interdependencies. In this study, two clinical criteria were evaluated to assess the caries activity status of occlusal surfaces in primary teeth. The authors did not assess the activity of cavitated lesions at the time of the examinations.

Caries risk assessment was determined using a modified CAMBRA (Caries Management by Risk Assessment) proposed by Featherstone et al. (2007). From the data collected, it was observed that the majority of the children (56%) were at moderate caries risk. The CAMBRA system represents an evidence-based approach to preventing, reversing, and treating dental caries (Young et al., 2007). This assessment occurs in two phases: the first is to determine specific disease indicators, risk factors, and

protective factors; the second is to determine the level of risk that the sum of these factors indicates (Featherstone et al., 2007). In the current study, the majority of lesions (72%) were clinically classified as potentially active. These results suggest an association between caries risk and caries activity status. It should be noted that most of the children of this investigation were from low-income families and their diet pattern was basically sugars and snacks.

Concerning the test sites, the worse status in each tooth was chosen in this study since the lesion can vary in severity across the occlusal surface (König, 1966). According to Jablonski-Momeni et al. (2009) clinical and laboratory studies have been assessing multiple sites on a single occlusal surface in order to increase the sample size. However, these studies have frequently been criticized for not having statistically independent data. It can be argued that the position and appearance of a lesion in one part of the fissure system could bias the judgment of the examiner about the appearance of a separate lesion in a different place in the fissure system and hence skew results.

Validating caries activity in a clinical study poses a number of problems, including sample selection, since only those teeth scheduled for extraction using other clinical criteria can be examined histologically (Ekstrand et al., 1998). Although the age of the patient in relation to tooth eruption pattern should be taken into consideration when evaluating the

caries active status (Ekstrand et al., 2009), this is reasonably difficult when teeth scheduled for extraction are used. Validation methods used have comprised histology with permeable dyes, histochemistry, electron microscopy, microradiography, microbiology, chemistry and biochemistry (Nyvad, Fejerskov, 1997). In the absence of a gold standard for caries lesion activity, predictive and construct validity can be used (Nyvad et al., 2003; Ekstrand et al., 2005).

As clinical changes assessed by visual inspection would be only detected by longitudinal studies to evaluate the progression of the lesions, dentists could not leave the caries lesions with no intervention in this period for ethical reasons. Therefore, over the years, many studies have proposed different diagnostic criteria based on a single visual inspection to assess activity of caries lesions (Ekstrand et al., 1998; Ekstrand et al., 2005; Nyvad et al., 1999; Quaglio et al., 2006). In the present investigation, caries lesions activity was evaluated based on clinical characteristics of the lesions in a single examination. For this purpose, the ICDAS-LAA criteria and the clinical visual/tactile criteria were tested. Our study showed strong correlation between both criteria, demonstrating that they are able to assess the caries activity status in the same way. A strong and significant correlation between the ICDAS-LAA and the Nyvad system for caries activity assessment was also seen by previous reports (Braga et al., 2009; Braga et al., 2010).

However, when the sample was split into noncavitated and cavitated lesions, there was no correlation between both systems among the cavitated lesions (Braga et al., 2010).

According to Nyvad and Fejerskov (1997), the criteria for the caries activity assessment should reflect the disease process and should be valid, reproducible and reliable. The ICDAS-LAA system has shown good reproducibility in predicting potential caries activity by recording the state of caries at one point in time (Ekstrand et al., 2007; Braga et al., 2009; Braga et al., 2010).

The CVT criteria are based on the evaluation of patho-anatomical changes due to caries. The clinical classification of caries activity status is not a new issue. In 1959, Miller described a detailed set of criteria based on the evaluation of clinical parameters, such as colour and consistency of surface layer, pain, age, progress and type of dentin under surface layer. However, identifying particular characteristics, such as dullness or shiny appearance, are very subtle changes and involve a relatively high degree of subjectivity (Ekstrand et al., 2009). In the present investigation, although the CVT criteria and the ICDAS-LAA present some differences to activity assessment and have not been compared before, they showed virtually identical performance, with no statistically significant differences. The results presented by the CVT criteria might indicate that the tactile sensation is associated with surface

roughness, as already described by Ando et al. (2010). In contrast, Ekstrand et al. (2005) reported poor accuracy when a clinical visual/tactile system was used to assess the activity status of carious lesions in children. This difference could be attributed to the visual/tactile criteria used, which assessed the visual appearance, the colour of the lesion and whether the lesion was dull/matt or shiny/glossy. Besides, in that pilot study, the authors determined whether dentists could differentiate between the appearances of lesions inactivated by regular professional oral hygiene and lesions which were not cleaned (active). It was observed that dentists were not able to reliably and reproducibly determine the subtle visual and tactile differences between active and inactive enamel lesions.

While the CVT criteria are based on the subjective observation that caries lesion activity is reflected in surface reflection and texture – chalky and rough lesions are active, and smooth, shiny, and hard lesions are inactive/arrested (Thylstrup et al., 1994), the ICDAS-LAA criteria use weighted numerical values for lesion appearance, lesion location in relation to a cariogenic plaque stagnation area and surface integrity (Varma et al., 2008). In this clinical study, the examinations were carried out by a well-trained examiner, after a special training session, which might have had an impact on in interpreting the clinical parameters for assessment of the caries

activity status. This can probably explain the comparable results between the two systems concerning caries activity evaluation.

The caries activity status is deduced from the behavior of the de- or remineralization of the dental tissue (Meller et al., 2006). During the caries process, calcium and other ions are released from tooth structure (Longbottom et al., 2008). Recently, a novel luminescence assay was developed to assess the caries activity status of caries lesions by a calcium-ion binder marker. The Carivis™ (LUX DS, Edinburg, UK) is a system based on the unique properties of the Glowdent™ (LUX DS, Edinburg, UK), a unique marker that captures minerals released during the dissolution of hydroxyapatite by acids generating a luminescent signal (Perfect et al., 2009). According to the manufacturer, Glowdent™ works in solution, and can be formulated as a liquid or gel. It can be applied to all tooth surfaces, including around restorations.

This study evaluated the validity of this novel assay for assessment of caries activity using the ICDAS-LAA and CVT methods as the validator. Results from this study showed a negative correlation between the luminescence activity and the ICDAS-LAA and the CVT criteria for caries activity assessment. It is important to point out that the luminescence analysis was not performed immediately after teeth exfoliation/extraction. In general,

the primary teeth were stored in 0.1% thymol solution for at least 4 months, and the storage media might have influenced the results.

According to Longbottom et al. (2008), in an in vitro study using primary and permanent teeth freshly extracted, luminescence signals were produced by all samples, principally from sites of visible caries and also from adjacent, apparently visually sound enamel. Other in vitro studies compared the results by the luminescence assay and the surface morphology using SEM methods in freshly extracted primary teeth. It was observed that the luminescence assay has the ability to assess caries activity, since regions of bright luminescence showed greater roughness than regions of low luminescence (Haughey et al., 2009; Perfect et al., 2009). These results show a potential ability of the novel luminescence assay to monitor and assess caries activity status.

Concerning the longitudinal data in the present study, it could be observed that both clinical criteria for caries activity assessment were similar when comparing the baseline data and the data collected after 2-month follow-up period in 10 primary teeth. Other studies also have been shown that ICDAS-LAA and Nyvad criteria have construct validity for caries activity assessment (Nyvad et al., 2003; Ekstrand et al., 2007). Despite the small number of teeth evaluated, these findings are notable considering that there are some differences between both criteria. Of the 10 lesions 2 appeared to

have remained active and 4 remained inactive. In addition, it was observed that 2 caries inactive lesions became active and 2 active lesions appeared to become inactive. It should be emphasized that the children did not receive any special treatment/maintenance since this was not the aim of this study. With regard to the luminescence signals captured after tooth exfoliation or extraction, they generally confirmed (7 out of 10) the caries lesions which were deemed to be arrested or active by the clinical assessment methods. However, luminescence signals demonstrated that 2 of the lesions clinically considered inactive at the second examination presented low signals of activity (an apparent false positive score) and 1 of the lesions clinically considered active at the second examination presented no signal indicating activity (an apparent false negative score). The false positive scores could in reality be true positive scores since the assay may produce light at a point in lesion development which is not discernible clinically. The fact that the ICDAS-LAA score had increased from 5 to 6 between the first and second examinations for both of these teeth suggests that these two lesions may well have been re-activating at a sub-clinical level. The false negative score may relate to an error in the clinical assessment – the 'true' activity status, being unknown. To date, as no validated reference method is available, further studies, using an appropriate validation method, are required in order to evaluate the performance of the luminescence assay to assess caries

activity. It is clearly important to determine which clinical criteria or method can assess caries activity most accurately.

In summary, from the present study the following can be concluded:

1. ICDAS-LAA and clinical visual/tactile criteria exhibited similar lesion activity scores and showed a high correlation when used to assess occlusal lesions in 88 primary molar teeth.
2. The luminescence assay provided signals from the majority of the sample teeth even after more than a year post-exfoliation/extraction, indicating the source of the signal, calcium ions in solution, was still present, despite long-term storage in thymol solution. The luminescence assay, though it had a negative correlation with the clinical activity assessments for all 88 teeth, 'correctly' assessed 7 of 10 sites which had been monitored longitudinally in vivo, using the clinical criteria as the reference standard.
3. ICDAS-LAA and clinical visual/tactile criteria assessed caries activity in a similar way after a 2-month follow-up period.

ACKNOWLEDGMENTS

Based on a thesis submitted to the graduate faculty, UNESP - Univ Estadual Paulista, Araraquara School of Dentistry, in partial fulfillment of the

requirements for the PhD degree of Michele Baffi Diniz. This investigation was partially supported by a scholarship from CAPES (#4053-08-7), Brazil, and by Indiana University School of Dentistry, USA. The authors want to thank LUX DS, Edinburgh, UK, for facilitating the luminescence analysis.

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Table 1. ICDAS criteria according to Ekstrand et al. (2007).

Code	Clinical criteria description
0	Sound tooth surface
1	First visual change in enamel
2	Distinct visual change in enamel
3	Localized enamel breakdown due to caries with no visible dentin or underlying shadow
4	Underlying dark shadow from dentin with or without localized enamel breakdown
5	Distinct cavity with visible dentin
6	Extensive distinct cavity with visible dentin

Table 2. ICDAS-LAA criteria according to Braga et al. (2009), adapted from Ekstrand et al. (2007).

Clinical parameter	Activity score	Description
Visual appearance	1	ICDAS score 1 or 2 (brown lesions)
	3	ICDAS score 1 or 2 (white lesions)
	4	ICDAS score 3, 4, 5 or 6
Plaque stagnation	3	Plaque stagnation area (entrance to pit and fissures, cavities with softened dentine)
	1	Non-plaque stagnation area (flat pits and fissures)
Surface texture	4	Rough or soft surface on gentle probing
	2	Smooth or hard surface on gentle probing

Table 3. Distribution of the clinical visual/tactile (CVT) and ICDAS-LAA activity scores with the luminescence assessments.

CVT/ICDAS-LAA activity	Luminescence signals				
	0	1	2	Total	p-value
0	5 (6%)	10 (11%)	10 (11%)	25 (28%)	0.8371
1	14 (16%)	20 (23%)	29 (33%)	63 (72%)	
Total	19 (22%)	30 (34%)	39 (44%)	88 (100%)	
CVT/ICDAS-LAA activity	Luminescence activity				
	0	1*	Total	p-value	Kappa
0	5 (6%)	20 (23%)	25 (28%)	0.8203	-0.02
1	14 (16%)	49 (56%)	63 (72%)		
Total	19 (22%)	69 (78%)	88 (100%)		

*Luminescence activity: Luminescence signals 1 and 2

Table 4. Spearman's rank correlation coefficients between all methods.

Methods	Correlation	p-value
CVT x ICDAS-LAA	1.00	
CVT/ICDAS-LAA x Luminescence signals	0.03	0.7866
CVT/ICDAS-LAA x Luminescence activity	-0.02	0.8223
Mantel-Haenszel chi-square test ($p<0.05$)		

Table 5. Specificity (Sp), sensitivity (Se) and accuracy (Ac) for the luminescence assessments at D_1 and D_2 thresholds considering the ICDAS-LAA or the CVT as the gold standard.

Method	D_1			D_2		
	Sp	Se	Ac	Sp	Se	Ac
Luminescence assessments	0.20	0.78	0.61	0.60	0.46	0.50

D_1: ICDAS-LAA or CVT scored as "active" and luminescence >0 = active caries lesion
D_2: ICDAS-LAA or CVT scored as "active" and luminescence >1 = active caries lesion

Table 6. Longitudinal data of the two criteria and the luminescence assay for caries activity assessment.

Tooth	1st Exam		2nd Exam		Luminescence signal
	ICDAS-LAA	CVT	ICDAS-LAA	CVT	
A	10	active	6	inactive	0
B	5	inactive	5	inactive	0
C	5	inactive	8	active	2
D	5	inactive	8	active	2
E	5	inactive	6	inactive	1
F	5	inactive	6	inactive	1
G	10	active	10	active	0
H	5	inactive	5	inactive	0
I	8	active	8	active	1
J	10	active	6	inactive	0

ICDAS-LAA: >7 = active caries lesion
Luminescence signal: >0 = active caries lesion

Figure 1. (A) Colour image as a reference to locate the test site as the circle marked on the occlusal surface (B) Black and white image of the same tooth. (C) Luminescence image using the “royal” colour scale captured after the addition of Glowdent™ (D) Luminescence image merged. Note: No luminescence signal is visible.

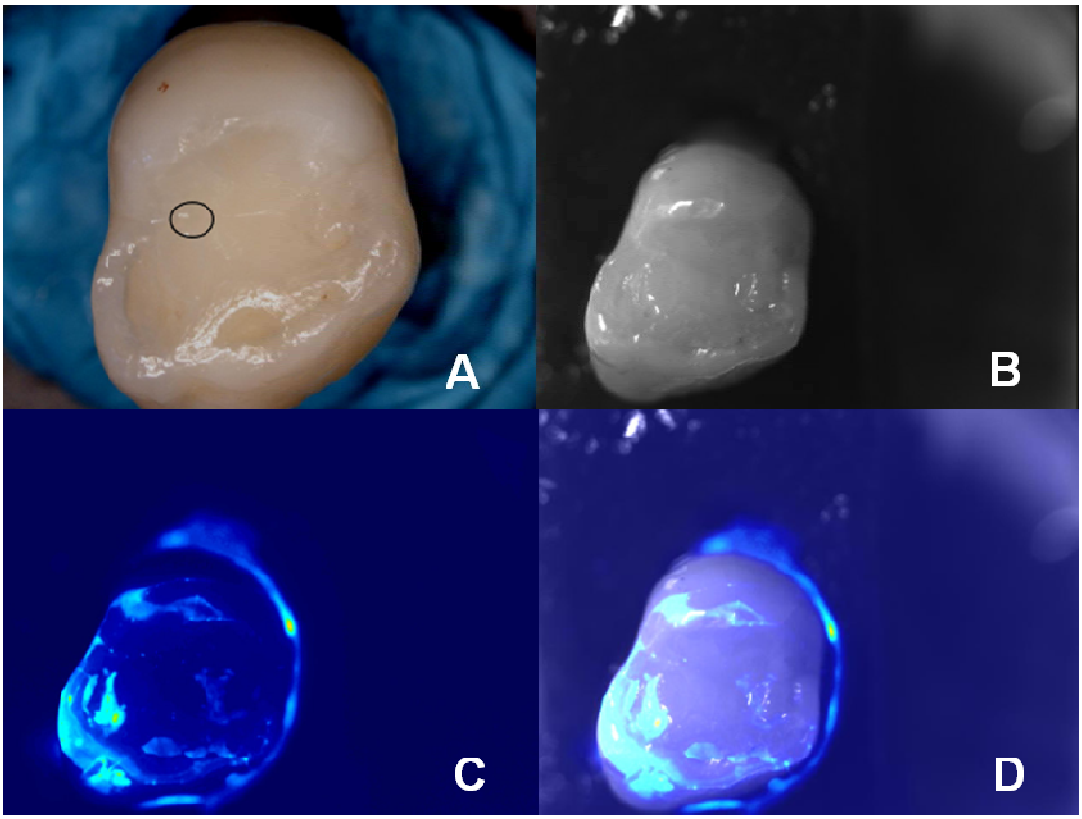


Figure 2. (A) Colour image as a reference to locate the test site as the circle marked on the occlusal surface (B) Black and white image of the same tooth. (C) Luminescence image using the “royal” colour scale captured after the addition of Glowdent™ (D) Luminescence image merged. Note: A dull blue luminescence signal.

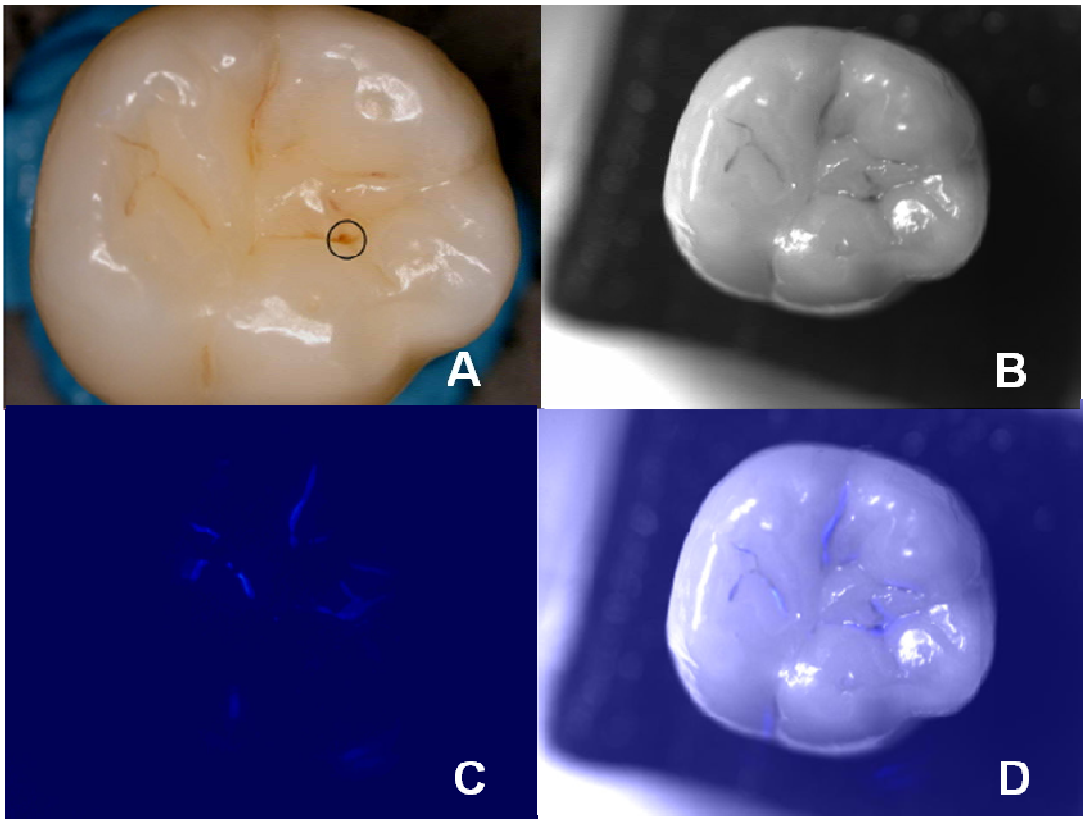
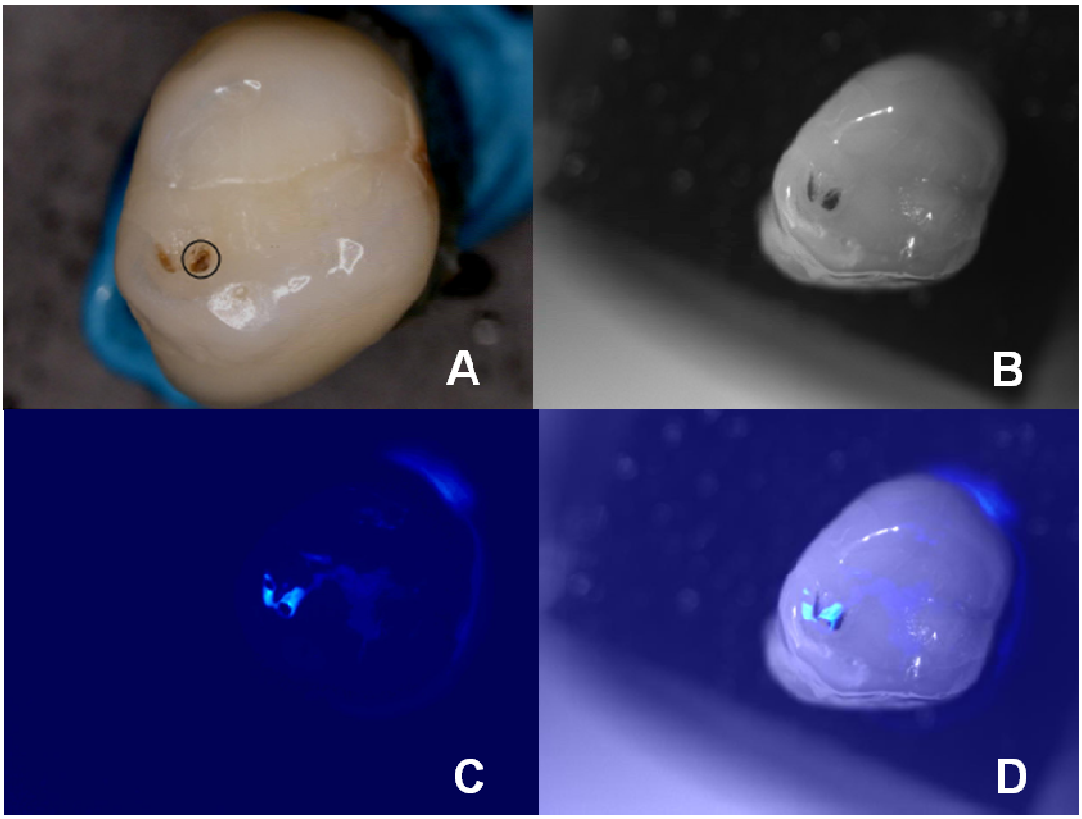
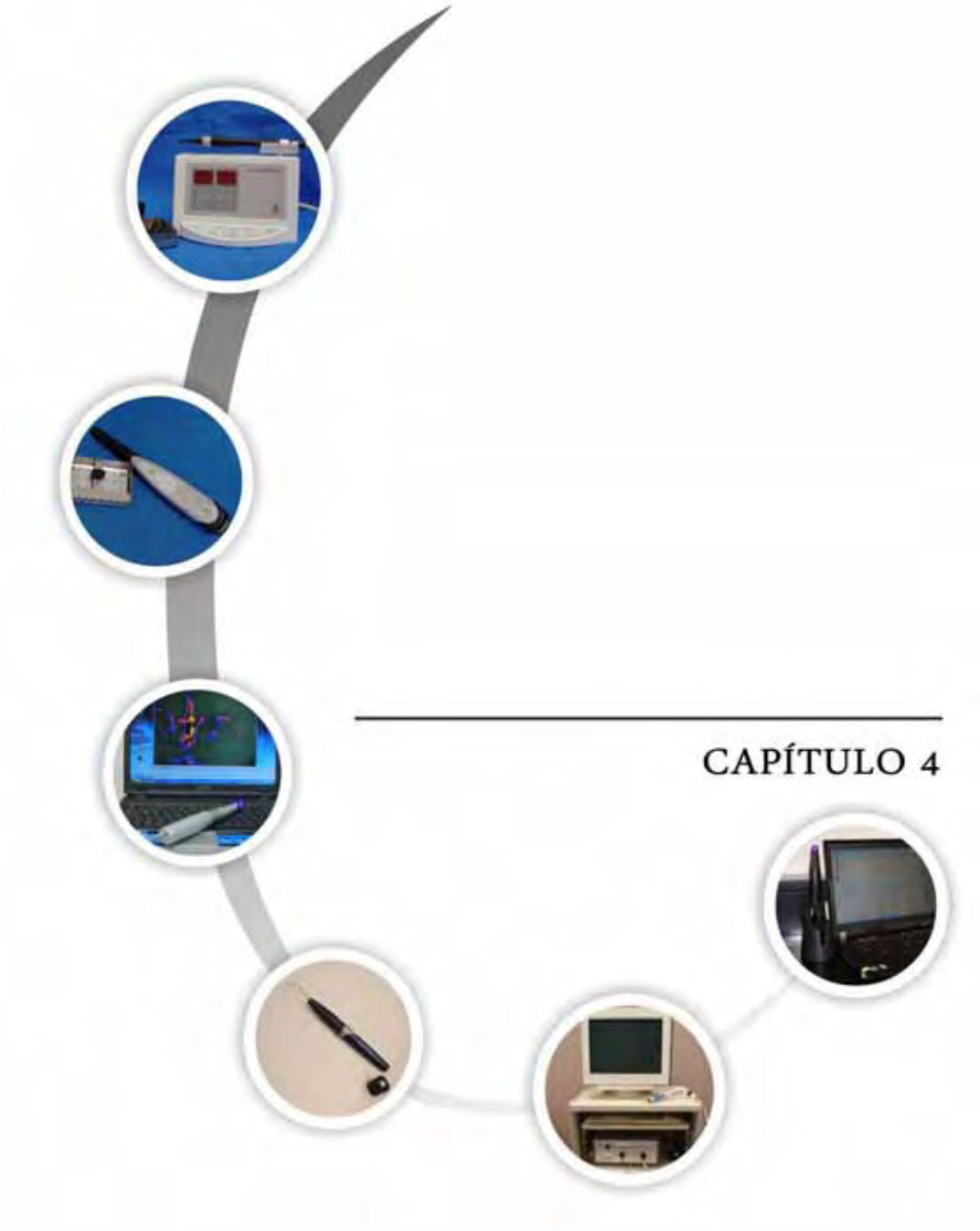


Figure 3. (A) Colour image as a reference to locate the test site as the circle marked on the occlusal surface (B) Black and white image of the same tooth. (C) Luminescence image using the “royal” colour scale captured after the addition of Glowdent™ (D) Luminescence image merged. Note: A bright luminescence signal.





CAPÍTULO 4

Evaluation of ICDAS and new technologies for detecting caries around amalgam and composite resin restorations

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Short title: Methods for detecting caries around restorations

Key-words: Occlusal caries, Secondary caries, Fluorescence, Visual examination, Marginal defect

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CLINICAL RELEVANCE

Caries around restorations is one of the most common reasons for replacing restorations. As accurate detection of those lesions is a difficult task in dentistry, new methods have been investigated to aid professionals during the clinical examination. In this investigation, the authors found that quantitative light-induced fluorescence and a novel method based on Fluorescence Enamel Imaging have potential to detect caries around amalgam and composite resin restorations. ICDAS visual criteria, tactile examination and laser fluorescence also presented good ability to detect caries around composite resin restorations.

SUMMARY

This in vitro study evaluated (1) the performance of the ICDAS visual criteria and new technologies for detecting natural caries around amalgam and composite resin restorations in permanent teeth, and (2) the relationship between the presence of caries around restorations and the presence/absence of different gap sizes. One hundred and eighty teeth with amalgam (group A; n=90) and composite resin (group B; n=90) restorations were selected. One occlusal site per tooth was chosen and marked on a photograph. Two examiners analyzed the teeth twice, with a one-week interval, using the ICDAS visual criteria (ICDAS), laser fluorescence (LF),

LED device (MID), quantitative light-induced fluorescence system (QLF) and a prototype system based on the Fluorescence Enamel Imaging (PCDS). Additionally, gap size was evaluated using an explorer probe. The teeth were sectioned through the selected sites and the gold-standard was determined by means of confocal laser scanning microscopy (CLSM). Intra-examiner repeatability and inter-examiner reproducibility was calculated showing high values for all methods (varying from 0.77 to 0.98), except for MID in the amalgam group (0.45). The performance was calculated using sensitivity, specificity, accuracy and the area under the ROC curve (Az). In the amalgam group, the QLF and PCDS were the most sensitive methods, while the other methods presented better specificity. Accuracy and Az values were 0.35/0.56 (ICDAS), 0.29/0.57 (LF), 0.30/0.55 (MID), 0.51/0.57 (QLF) and 0.57/0.57 (PCDS), respectively. In the composite resin group, QLF and PCDS were the most sensitivity methods, while the other methods presented higher specificity. Accuracy and Az values were 0.72/0.75 (ICDAS), 0.74/0.82 (LF), 0.54/0.65 (MID), 0.74/0.82 (QLF) and 0.72/0.73 (PCDS), respectively. Regarding the relationship between the gap size and the presence of caries around restorations, caries was found in 48% and in 30% of the amalgam and composite resin restorations, with intact margins, respectively. In conclusion, QLF and PCDS appear as potential methods for detecting caries around amalgam and composite resin restorations. ICDAS and LF also presented

good ability to detect caries around composite resin restorations. Gap size was found to be irrelevant to determine the presence of caries around restorations.

INTRODUCTION

Caries around restorations (CARS), commonly called “secondary caries”, represents the major reason for the replacement of amalgam and composite resin restorations in operative dentistry.[1-4] CARS indicates a lesion which occurs at the margins of an existing restoration.[4,5] The lesion has classically been described as occurring in two areas: an “outer lesion” formed in the enamel, cementum, or dentin of the tooth surface, similar in histology and etiological factors to a primary lesion; and a “wall lesion”, which is a narrower defect in the enamel and/or dentin along the cavity wall restoration interface produced by leakage of bacteria, fluids, molecules or hydrogen ions between the restoration and cavity.[1]

It has been recognized that these lesions are extremely difficult to detect clinically due to stain around a tooth-colored filling or the presence of residual caries, unless they are in an advanced stage or become cavitated.[6-8] Although the tactile examination is not an accurate method, it is still the most widely used method associated with visual inspection for the detection of caries around restorations.[9] Another important issue in daily dental practice is

related to the defective margins of the restorations. For many years, it has been assumed that marginal defects act as a gathering point for bacterial plaque, which leads to the development of caries around restorations.[10] Some authors have shown a positive correlation between increasing gap size and the presence of caries around restorations,[11-15] while some others have found contradicting results.[6,16,17] To date, this issue remains unresolved.

Visual inspection is based on subjective criteria, such as color, translucence and dental hardness,[18] having high specificity and low sensitivity.[19] Recently, the International Caries Detection and Assessments System (ICDAS) was introduced as a new visual criterion for caries detection. The system proposes seven codes for caries detection, varying from 0 to 6 based on lesion severity. Some studies have shown good reproducibility and accuracy of ICDAS for occlusal caries detection in permanent teeth.[20-22] Since outer lesions are considered similar to primary caries, the principles applied to the criteria for primary caries are also applied to caries around restorations and sealants (CARS).[23] A preliminary study found that ICDAS presented excellent reproducibility and correlated well with other methods for detection of caries around amalgam restorations.[24] However, it should be stressed that there is no published study evaluating the performance of the ICDAS criteria for the detection of caries around restorations.

Technology based early caries detection methods have been receiving considerable attention as an aid for detection and quantification of caries around restorations.[9] The infrared laser fluorescence device (LF, DIAGNOdent 2095, KaVo, Biberach, Germany) is capable to capture fluorescence emitted by oral metabolites (porphyrins) when the dental tissue is stimulated by a red light laser with 655nm of wavelength.[25] Some studies have shown moderate to good performance of the laser fluorescence device to detect caries around restorations.[26-29]

Recently, another device for caries detection was developed based on LED technology (MID, Midwest Caries I.D., DENTSPLY Professional, York, PA, USA). The device emits a soft light emitting diode (LED) between 635 nm and 880 nm and analyzes the reflectance and refraction of the light from the tooth surface, which is captured by fiber optics and is converted to electrical signals for analysis. The microprocessor of the device contains a computer-based algorithm that identifies the different optical signature (changes in optical translucency and opacity) between healthy and demineralized tooth.[30] The demineralization leads to a change in the LED from green to red with a simultaneous audible signal, which is directly related to the severity of caries lesions. There are few preliminary studies regarding its performance for occlusal and proximal caries detection.[31-33] Although false positives with MID

can be caused by the presence of restorations,[24] it is important to test its ability for the different clinical situations.

Quantitative light-induced fluorescence (QLF) (QLF-clin, Inspektor Research Systems BV, Amsterdam, The Netherlands) is a system aiming to monitor changes in mineral content of enamel. This device consists of an intra-oral camera and a portable arc-lamp unit with 290 and 450 nm of wavelength and it is connected to a computer. A specific software allows capturing the fluorescent images and posterior analysis.[34] It has been shown to allow detection of caries around restoration.[9,35-38] A similar system based on fluorescence was developed to detect, quantify and monitor early caries lesions.[39] The FluoreCam system (Daraza, Corporate Headquarters, Noblesville, IN, USA) works on the same principle as the QLF system, the main difference being the software which automatically finds and outlines the caries lesion for analysis.[40] This device has been initially tested in the USA as PCDS - Professional Caries Detection System (Therametrics Technologies, Inc., Indianapolis, Indiana, USA). However, to date, there are no published studies concerning its ability to aid caries detection.

The difficulty in differentiating among caries around restorations, marginal defects and residual caries has resulted in uncertainty and confusion by the clinical practitioners calling for studies to increase the knowledge and improve diagnostic criteria for caries around restorations.[41]

Thus, the aims of this in vitro study were (1) to evaluate the performance of the ICDAS visual criteria, laser fluorescence (LF), LED device (MID), quantitative light-induced fluorescence system (QLF) and a prototype system based on the Fluorescence Enamel Imaging (PCDS) for detecting natural caries around occlusal amalgam and composite resin restorations in permanent teeth, and (2) to determine the relationship between the presence of caries around restorations and the presence/absence of different gap sizes.

METHODS AND MATERIALS

Sample Selection

The research protocol was approved by Local Ethics Committee in Araraquara, São Paulo, Brazil (Protocol 48/08). One hundred and eighty human permanent posterior teeth with class I amalgam restorations (Group A; n=90) and composite resin restorations (Group B; n=90) were selected from a pool of extracted teeth obtained by dental practitioners in Brazil and stored in 0.1% thymol solution for up to three months. The selected teeth ranged from having intact margins to cavitated margins on the occlusal surfaces.

The teeth were cleaned with water and a disposable prophy angle attached to a low-speed handpiece. Calculus and debris were removed using

a scaler. Afterwards, the teeth were identified and individually stored in plastic containers containing 0.1% thymol solution at 4℃ until use. Photographs of the occlusal surfaces were taken using a stereomicroscope (Nikon SMZ1500, Nikon, Tokyo, Japan) with magnification of 10x, equipped with a digital camera (Nikon DXM1200, Nikon, Tokyo, Japan) and an imaging software (Nikon ACT-1, version 2.62, Nikon, Tokyo, Japan).

One examiner selected one occlusal site per tooth at the tooth-restoration interface cavosurface based on the presence or absence of gap using an explorer probe with 0.1 mm diameter tip under a stereomicroscope with magnification of 10x. The test sites were marked with an opaque dot and the images printed.

All assessments were carried out twice by two independent experienced examiners, observing a one-week interval between the measurements. Prior to the examination, all examiners participated in a discussion and training of the different methods for caries detection during a 4-hour session.

Teeth were mounted in twelve plastic dental models (upper and lower arches) (Nissin Dental Products, Inc., Kyoto, Japan) using base wax and stored at 100% humidity with 0.1% thymol solution to prevent bacterial growth. The models were placed in a phantom head (Kilgore International,

Inc., Coldwater, MI, USA) to simulate, as closely as possible, conditions similar to patient's oral examination.

Visual Examination

The teeth were assessed by the examiners following the ICDAS criteria for caries associated with restorations and sealants (CARS) as suggested by the ICDAS Coordinating Committee (Table 1). The examinations were performed in the same room, under good illumination, using a portable dental light, and aid of a 3-in-1 air syringe, a dental mirror and a periodontal index probe. The teeth were analyzed moist and then dried with no magnification.

Laser Fluorescence

The test sites were evaluated using a laser fluorescence device (LF, DIAGNOdent 2095, KaVo, Biberach, Germany) according to the manufacturer's instruction. For the measurements, the tapered fiber optic tip A was used. Initially, the device was calibrated before every measurement against the ceramic standard, and then, calibrated on a sound central area of the buccal surface of each tooth. The teeth were air dried and the tip was placed on the test site and rotated around a vertical axis. The highest value (peak value) was recorded. Any measurements >8 were considered to be

carious.[9] Fluorescence in the center of the surface of the restoration was also measured and recorded.[26]

LED Device

The teeth were also analyzed using a new LED device (MID, Midwest Caries I.D., DENTSPLY Professional, York, PA, USA), according to the manufacturer's instructions under wet conditions. After calibration in the ceramic calibration target through the process of measuring a baseline optical response in wet conditions, the probe was placed in direct contact in the long axis of the occlusal area near the margins of the restoration, with no pressure against the tooth surface. During probing, the handpiece emitted an audible signal (from slow to rapid) associated with a visual signal (green indicates sound surface and red indicates demineralization) in order to differentiate the presence and extent of caries lesions. The audible signals were recorded as: (0) no signal, (1) slow, (2) moderate, and (3) rapid.

Quantitative Light-Induced Fluorescence System

Fluorescence images were taken using QLF device (QLF/clin 007, Inspektor Research Systems BV, Amsterdam, The Netherlands). After carefully drying, the teeth were illuminated by a violet-blue light ($\lambda \approx 370 \pm 80$ nm), and the fluorescent images were filtered through a 520 nm high-pass

filter on a computer monitor in a completely darkened conditions to reduce extraneous light.[9] The handpiece with a dental mirror was fixed under a right-angled above each tooth and the image was collected, stored and analyzed live on the screen using QLF Patient software (version 3.0.0.35, Inspektor Research Systems BV, Amsterdam, The Netherlands). In this study, a visual ranked scale was used by the examiners observing live images on the screen according to Ando and others[9]: (0) no change in enamel fluorescence, (1) slight change in enamel fluorescence, (2) fluorescence loss distinctly visible without enamel broken, (3) fluorescence loss distinctly visible with enamel broken, and (4) fluorescence loss distinctly visible with cavitation.

Fluorescence Enamel Imaging System

Fluorescence images were also taken using the Professional Caries Detection System (PCDS, Therametrics Technologies, Inc., Indianapolis, IN, USA), a prototype of a new portable instrument based on Fluorescence Enamel Imaging technology (FluoreCam, Daraza, Corporate Headquarters, Noblesville, IN, USA). Teeth images were taken in a dark room and analyzed live on the screen by specially designed software (FluoreCam version 1.2.3.0., Therametrics Technologies, Inc., Indianapolis, IN, USA). The same ranked scale described for the QLF was used by the examiners to evaluate the fluorescence images.

Gap Evaluation

Gap evaluation was performed by one experienced examiner after all examinations by probing gently the test site with the tip of an explorer probe (0.1 mm in diameter tip) to evaluate the marginal gap size at the tooth-restoration interface. Gaps were classified using the following scores as proposed by Moazin[10]: (0) intact margin (no visible gap or <0.05 mm), (1) small marginal gap (between 0.05 and 0.1 mm, explorer tip does not go through the tooth-restoration interface), and (2) large marginal gap (>0.1 mm, explorer tip goes through the tooth-restoration interface).

The accuracy of the gap size classification was confirmed by measuring the gap size of all teeth in digital images taken with a digital stereomicroscope (Nikon SMZ1500, Nikon, Tokyo, Japan) with magnification of 30x equipped with a digital camera (Nikon DXM1200, Nikon, Tokyo, Japan) and an imaging software (Nikon ACT-1, version 2.62, Nikon, Tokyo, Japan).

Confocal Laser Scanning Microscope (CLSM) Analysis

After examinations, the teeth were sectioned through the investigation sites using a water-cooled hard-tissue sectioning machine (Gillings-Hamco, Hamco Machines, Inc., New York, NY, USA). The specimens were then stained with 0.1 m*M* buffered (pH 7.0) rhodamine B solution (Aldrich Chemical Company, Inc., Milwaukee, WI, USA) for 24 h. The specimens were

allowed to air-dry for 1 h and then analyzed with a confocal laser scanning microscope (Zeiss LSM 510 Meta Confocal, Carl Zeiss Optical, Inc., Thornwood, New York, NY, USA). This 2-photon microscope system uses a helium/neon laser with 543-nm excitation wavelength, a 25μm confocal slit, a 550-m-long pass barrier filter, and a 10x objective lens. CLSM settings were optimized and standardized for all specimens. Images were obtained plano-parallel to the transversal cut surface of the specimen and perpendicular to the natural surface of the tooth. Then, the digital images were analyzed using the Metamorph software Program (version 4.0, Universal Imaging Co., Dowing Kown, PA, USA) to assess the carious lesion extension in enamel and dentin at the area showing the maximum lesion depth (greatest penetration of the Rhodamine B dye). In cases where the wall and outer lesions were continuous, an imaginary line was drawn to bisect the cavity wall angle into two equal halves separating the wall and outer lesion areas. The lesion measurement was performed as: the outer surface lesion was measured as the largest distance between the enamel surface and the inner border of the lesion. The wall lesion was measured as the distance between the restoration and the inner border of the lesion. Measurements of the outer and wall lesions were done independently. At the same time, any physical connection between outer and wall lesions was recorded to assess the relation in lesion development between both the outer and wall lesions. Then,

the maximum of the wall and outer lesion depths was used as the severity ranking scale for each specimen using a newly developed scoring system proposed by Moazin[10], as presented in Table 2.

Statistical Methods

All analyses were performed separately for amalgam and resin restorations. For the comparisons of the methods, data from both examiners were used. The data were decoded and entered into Excel and then exported to SAS (version 9.2, SAS Institute, Inc., Cary, NC, USA) for statistical analysis. Average and standard deviation of LF fluorescence values were calculated for each group.

Intra- and inter-examiner repeatability of methods was assessed using intra-class correlation coefficients (ICCs). ICCs were used rather than kappa statistics to account for the intra-examiner repeatability in the inter-examiner agreement calculation.

Specificity, sensitivity, accuracy and area under the ROC curve were calculated for each method considering overall caries lesions. The closer the ROC curve area is to 1.0, the better the diagnostic test. For this calculation, the definitions were caries if CLSM>0 and any scores >0 for all methods. The ROC curve indicates a generally consistent tradeoff between sensitivity and specificity if the cut-off were to be moved higher or lower. Sensitivity values were also calculated for each method at D_1, D_2 and D_3 thresholds for

detecting lesions of different sizes once there is a lesion. These computations were carried out using three different cutoffs: caries if CLSM=1 (D_1), caries if CLSM=2 (D_2), and caries if CLSM=3 (D_3). Comparisons between methods were performed using bootstrap analyses. The bootstrap methodology uses resampling techniques to estimate statistics and perform comparisons for values that are not normally distributed. In this case the bootstrap also provided a way to properly account for the correlations between examiners, between repeats, and between the methods.

Spearman correlation coefficients were calculated to measure the associations of the methods with CLSM depth, and also of the accuracy of the gap size with the other methods. Significance of the Spearman correlation coefficients was testing using bootstrap methods to similarly account for data from multiple examiners and repeats within examiner.

RESULTS

From the 90 occlusal test sites analyzed in the group A, the CLSM depth analysis showed that 39 (43%) teeth had no lesions, 15 (17%) had small lesions, 27 (30%) had medium lesions, and 9 (10%) had large lesions. From the 90 occlusal sites in the group B, 28 (31%) teeth had no lesions, 6 (7%) had small lesions, 25 (28%) had medium lesions, and 31(34%) had large lesions.

The mean ± standard deviation of LF peak measurements in the test site and in the center of the restoration of all teeth were, respectively, 4.0 ± 11.9 and 0.2 ± 0.6 in the group A, and 17.1 ± 22.1 and 4.3 ± 4.7 in the group B.

Intra- and inter-examiner reproducibility for all methods is shown in Table 3. ICCs values were acceptably high, varying from 0.77 to 0.98, indicating substantial agreement for all methods in both groups, except for the MID device, which showed lower reproducibility (0.45) in the group A.

Specificity, sensitivity, accuracy and area under the ROC curve for all methods considering overall lesions are shown in Table 4. In the group A, ICDAS (0.70), LF (0.84) and MID (0.87) presented significantly higher specificity when compared to the QLF and PCDS systems. However, QLF and PCDS presented the highest sensitivity (0.61 and 0.71, respectively) and accuracy (0.51 and 0.57, respectively) values. The area under the ROC curve (Az) was moderate for all methods, with no statistically significant difference (varying from 0.55 to 0.57). In the group B, ICDAS (0.73), LF (0.83) and MID (0.92) also presented significantly higher specificity, while QLF and PCDS presented the highest sensitivity values (0.84 and 0.79, respectively), followed by ICDAS (0.71) and LF (0.70). Accuracy values were moderate for all methods (ranging from 0.72-0.74), except for the MID device, which showed significantly lower accuracy value (0.54). The Az values were

moderate to high (varying from 0.73 to 0.82) for all methods, expect for lower value presented for the MID device (0.65).

Sensitivity values at D_1, D_2 and D_3 thresholds are shown in Table 5. In the group A, at D_1 threshold, QLF was found to have the highest sensitivity, followed by PCDS. At D_2 and D_3 thresholds, higher sensitivity values were demonstrated by PCDS (0.72 and 0.74, respectively) and QLF (0.64 and 0.58, respectively), with no statistically significant difference between them. The other methods presented relatively lower sensitivity values. In the group B, moderate sensitivity was achieved by QLF (0.51) and PCDS (0.50) at D_1 threshold, followed by ICDAS and LF. ICDAS, LF, QLF and PCDS showed higher sensitivity values, with no statistically significant difference among them at D_2 threshold, while MID showed the lowest sensitivity value (0.25). At D_3 threshold, all methods presented high sensitivity values, varying from 0.77 to 0.97, except the MID device, which presented a sensitivity value of 0.55.

Table 6 presents the correlations between all methods and CLSM depth analysis in both groups. Spearman coefficients were moderately correlated for the group B, ranging from 0.51 to 0.65, while were uncorrelated in the group A.

Table 7 shows Spearman correlation coefficients between the accuracy of the gap size determined by the measurement of the gap size in digital images and the other methods. In the group B, a moderate correlation

was found with all methods. There was a marginally significant relationship between gap size and ICDAS, QLF and PCDS. However, in the group A, the methods were weakly correlated with the gap size, except for the ICDAS criteria.

Cross-tabulation between the accuracy of the gap size and the CLSM lesion depth is presented in Table 8. In the group A, 19% of the teeth with no demineralization visible on the CLSM analysis had no gap, while in the group B, 27% of the teeth had no caries and no gap. CLSM analysis revealed caries in 48% of the intact margins, and in 16% and 6% of the margins with small and large gaps, respectively. In the group B, 30% of the intact margins presented caries, and 21% and 18% of the margins, with small and large gaps respectively, presented caries.

DISCUSSION

As caries around restorations are difficult to detect clinically, new detection methods would allow early detection, and implementation of preventive strategies to reduce future need for replacement of restorations.[41] In the current study, the potential of different methods for detecting natural occlusal caries around restorations was investigated. Although it has been recognized that caries around restorations is more prevalent in the gingival margin of all types of restorations[41], several studies has been confirming its

occurrence in occlusal surfaces, where fissures might left untouched during cavity preparation, allowing the development of caries.[16,42.43]

In our study, caries around two types of restorative materials (amalgam and composite resin restorations) were evaluated, since some factors related to the dental materials may predispose the margins of restorations to develop caries lesions. Caries around restorations was seen more often on the occlusal part of composite resin restorations (69%) than on the amalgam restorations (57%), which is in accordance with previous reports.[42,43]

In the present investigation, the extension of caries around amalgam and composite resin restorations was determined with the CLSM analysis to measure the depth of lesion fluorescence. There are several methods available to evaluate mineral loss in caries around restorations as gold standard, such as histopathology[26], polarized light microscopy[6,44], transverse microradiography[45], clinical examination of cavity and tactile consistency using caries-detector dye[29] or not[28] and confocal laser scanning microscopy.[9,36] In this study, CLSM was used because it does not require special specimen preparation processing, the evaluation can be performed under environmental condition, and it is a not time-consuming technique.[46]

Regarding the reproducibility, the examiners demonstrated high agreement in all methods for both groups, except for the MID device in the

group A. The substantial agreement could be explained due to the experience and additional training of the examiners. The excellent reproducibility is in line with other in vitro studies evaluating the ICDAS[24], the LF device[9,24,26] and the QLF system[9] for detecting caries around restorations. However, the moderate agreement in MID could be due to the system and/or individual mistakes in operating the device, and due to influence of the amalgam material. Similar results were also described in a previous study to detect occlusal caries around amalgam restorations.[24]

It could be observed that each method evaluated in this study showed different validity for detecting caries around restorations. It must also be recognized that the visual examination of caries around restorations is not a well-defined entity, in view of the fact that the enamel lesion color can vary depending upon the adjacent restorative material. While the outer lesion can be seen as a white or brown spot lesion with or without softening of the mineralized tissues, or a frank cavitation, the wall lesion is not easy seen until demineralization is sufficiently advanced to shine up through the overlying enamel or the latter collapses to show cavitation.[1] Within the limitations of an in vitro study, the examinations were performed in order to simulate a clinical examination in terms of adjacent tooth and gingivae, although was not impaired by saliva and plaque. Based on the results, overall analysis of sensitivity and specificity values showed that ICDAS visual criteria presented

higher specificity (0.70) and lower sensitivity (0.20) and accuracy (0.35) for detecting caries around amalgam restorations (group A). A previous study also found similar results when using the visual criteria proposed by Ekstrand and others[18] to detect caries around amalgam restorations.[9] The lower sensitivity could be explained by the dark coloured corrosion products formed around amalgam restorations. For this reason, more staining/discolouration was expected which might present a diagnostic difficulty.[1] Lower sensitivity was also described by Bamzahim and others[28], although they used a different criterion for the visual examination in an in vivo study concerning caries around amalgam restorations. At the different thresholds, the sensitivity values decreased significantly.

On the other hand, ICDAS visual criteria showed higher specificity (0.73), sensitivity (0.71) and accuracy (0.72) values for detecting caries around composite resin restorations (group B). These findings agree with previous in vitro results related to the visual examination to detect caries around composite resin restorations in permanent teeth.[26] Although the differentiation between caries around tooth-colored materials and marginal staining is an inherent problem during diagnosis[47], color changes may indicate early caries adjacent to a tooth-coloured restoration. Those signals may represent a white or brown spot lesion around a filling, a grey discolouration from demineralized dentin deep to the enamel cavity wall, or a

line of stain at the junction of the filling and the tooth.[1] In respect with the different CLSM thresholds, the ICDAS visual criteria presented higher sensitivity when the carious lesions depth was medium (D_2) and large (D_3), which disagrees with Boston[26], who observed lower sensitivity value (0.45) for the visual criteria to detect dentin caries around composite resin restorations.

It is known that LF readings can be influenced by the presence of dental materials, which have a different fluorescence signature from enamel, giving false results. In our study, when measured in the center of the amalgam and composite resin surfaces, all restorations produced little fluorescence after calibration of the LF device, up to a maximum reading of 9. However, Boston[26] observed LF readings in the center of composite resin restorations up to 22 units. These differences could be attributed to the teeth storage media used in that study (formalin or 2.5% glutaraldehyde), which probably affected the LF readings. Although teeth stored in formalin, chloramine and thymol showed a statistically significant decline in LF readings over 2 years[48], in our study, the teeth were stored in 0.1% thymol solution at low temperature conditions for only few months in view of the fact that it is a common antiseptic solution to avoid bacterial growth.

Although some in vitro studies have been demonstrating a potential ability of the LF device for detecting caries around amalgam restorations[9,28,29], in the present study the LF device showed higher specificity

and lower sensitivity and accuracy values. Lower sensitivity was also observed at the different CLSM thresholds, which also disagrees with previous results.[9] Higher specificity of the LF device was also showed by an in vivo investigation.[28] In contrast, LF presented high specificity, sensitivity and accuracy values for detecting caries around composite resin restorations. This finding agrees with a previous study regarding the detection around resin restorations.[26] Other studies have also shown that LF is able to detect lesions associated with composite resin restorations.[37,38,4]. It could also be observed, as the CLSM threshold increased, sensitivity values of LF device also increased for lesions depth over 100 µm (D_2 and D_3). It is important to point out that the recommendation for detection of caries around restorations cannot be based on the LF values alone because sound and carious sites are represented by overlapping ranges of LF values.[27]

The MID device has been shown to be effective for both occlusal and proximal caries detection on a wet condition (Strassler and Sensi, 2008). Even though this new method could represent a useful tool to aid the diagnostic process, this device can be negatively influenced by the presence of restorations.[30] A preliminary study found that MID was weakly or not correlated with LF and QLF to detect caries around amalgam restorations.[24] Our results showed high specificity values for MID in detecting caries around amalgam and composite resin restorations. However, lower sensitivity values

were also found, including at the three different CLSM thresholds for both groups, indicating its poor ability in detecting caries around restorations, which confirms the signals interference caused by the dental materials, and that its use off label is not appropriate.

Despite previous descriptions of QLF analysis as a complicated method to be applied for detecting caries around restorations[9], prior studies have shown a potential ability of this method for detection or early caries lesions adjacent to amalgam and composite resin restorations.[37,38,49] In the present study, the QLF analysis was performed using a visual ranked scale to determine the presence or absence of demineralization adjacent to restorations. This technique was previously evaluated by other authors and showed good results.[9,49] Based on our results, QLF was found to have the lowest specificity and the highest sensitivity and accuracy values among the techniques to detect caries around composite resin restorations, which disagree with previous results.[38] This difference could be attributed to the gold standard method used by them, which was a subjective visually/tactilely examination of the cavities after restoration removal. In addition, in the same group, the sensitivity values at the three CLSM thresholds increased proportionally with increasing lesion depth. For the amalgam restorations group, the specificity was low and the sensitivity was high. Similar results were also described by Ando and others.[9] At the different thresholds, the

sensitivity values decreased moderately with the increasing lesion depth, which confirms the difficulty in analyzing demineralization around amalgam restorations due to the dark fluorescence images obtained with this type of material.

The FluoreCam, a novel Fluorescence Enamel Imaging (FEI) technology, has been recently introduced as an innovative and revolutionary instrument that detects and quantifies early caries lesions.[39] In this study, a prototype (PCDS) of this system was evaluated to detect caries around restorations. The performance of the PCDS was similar to the QLF, which showed low to moderate specificity and high sensitivity to detect caries around amalgam and composite resin restorations. At the different CLSM thresholds, PCDS presented similar sensitivity values when compared to the QLF device in both groups. These findings were not surprising as both systems are based on the same principle of tooth fluorescence. During the examinations, it was possible to observe that PCDS images were easier to capture. This fact could be attributed to the specific instrument tip, which allows fixing the camera's focal point adjacent to the tooth surface.[40] However, it could be observed that the images visually appear to have lower resolution than the QLF images.

The area under the ROC curve (Az) showed the poor performance of the methods, with no statistical significant difference, in detecting occlusal

caries around amalgam restorations. Ando and others[9] also observed moderate Az values for the visual examination and both LF and QLF devices. However, the LF device presented higher Az values in previous studies.[27-29] In the composite resin group, the LF and the QLF devices presented the highest Az values, although there was not statistically significant different from the ICDAS and PCDS Az values. Boston[26] also observed higher Az values for LF and visual examination to detect caries around composite resin restorations.

The non-correlation between the methods and the CLSM lesion depth confirmed the difficulty in detecting caries around amalgam restorations. This indicates that the access of the methods tested may be hindered by the amalgam materials. Besides it is tricky to differentiate between caries around amalgam restoration and staining. However, the methods showed good and significant correlation to detect caries around composite resin restorations, suggesting their potential to detect early demineralization. It must be stressed that alterations can be more easily recognized around tooth coloured restorations than around amalgam restorations.

Another important aspect related to the difficulty in detecting caries around restorations is related to the marginal integrity, since it is one of the principal criteria used for judgment of restoration quality.[6] Whenever a restorative material is placed, there is a possibility for a microspace (gap) to

be formed between the restorative material and the cavosurface enamel, dentin, and cementum. Thus, the lack of marginal integrity and sealing increases the risk of caries around restorations over time. However, it must be emphasized that not all leaky restorations with marginal defects could lead to the development of caries lesions.

Correlation observed in this study between accuracy of the gap size and ICDAS visual criteria were moderate for both groups. This finding might indicate an association between the presence of gaps, crevices, and ditches that exhibit characteristic changes associated with caries, such as softening tissues, color changes, and/or cavitation, and consequently, indicating the appropriate intervention strategy.[41] In addition, a moderate correlation was found between gap size and the other methods for the composite resin restorations group, while lower correlation was observed for the amalgam restorations group.

Several studies have evaluated the importance of gap size in the formation of caries around restorations. In this in vitro study, the relationship between the presence of caries around restorations and the presence of different gap sizes at the enamel-restoration interface was investigated. The results observed strongly suggest that there is not a relationship between an increase in gap size and the presence of caries around restorations. In other words, caries was found in 48% and in 30% of the amalgam and composite

resin restorations, with intact margins, respectively. Our findings are in accordance with previous studies, which also found caries when the restorations margins are clinically sound.[6,16] In contrast, Jorgensen and Wakumoto[11] observed that caries was not found in gaps smaller than 35-50 µm in occlusal amalgam restorations.

It is known that the management of caries around restorations involves determining some risk factors to control its progressions.[1] In this study natural caries was investigated instead of artificial caries induced around restorations using extracted teeth. However, this type of study has some limitations such as unknown history of the restorative procedure, patient's age and sex, oral hygiene, fluoride use, personal diet, which are important aspects related to the development of caries around restorations. Also, the impossibility to differentiate between residual caries, which may have been left during cavity preparation, and caries around restoration must be inferred. Although the implications in this study do not provide evidence for clinical application they can show significance for future investigations.

CONCLUSIONS

On the basis of these results, it can be concluded that QLF and PCDS appear to be potential methods for detecting caries around amalgam and composite resin restorations. It seems that ICDAS criteria are difficult to

distinguish between incipient caries around amalgam restorations from staining or ditching. However, ICDAS and LF presented good ability to detect caries around composite resin restorations. Besides, the presence of gap by itself was not a strong factor to determine the presence of caries around amalgam and composite resin restorations.

ACKNOWLEDGEMENTS

Based on a thesis submitted to the graduate faculty, UNESP - Univ. Estadual Paulista, Araraquara School of Dentistry, in partial fulfillment of the requirements for the PhD degree of Michele Baffi Diniz. This investigation was partially supported by a scholarship from CAPES (#4053-08-7), Brazil, and by Indiana University School of Dentistry, USA. The authors want to thank Brigitte Marie Dan for helping to obtain the images with the quantitative-light induced fluorescence system and Amir Haider, for the assistance during CLSM analysis.

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Table 1. ICDAS criteria for caries associated with restorations and sealants (CARS) according to the ICDAS Coordinating Committee.[50]

Code	Clinical criteria description
0	Sound tooth surface with restoration or sealant
1	First visual change in enamel
2	Distinct visual change in enamel/dentin adjacent to a restoration/sealant margin
3	Carious defects of <0.5 mm with the signs of code 2
4	Marginal caries in enamel/dentin/cementum adjacent to a restoration/sealant with underlying dark shadow from dentin
5	Distinct cavity adjacent to a restoration/sealant
6	Extensive distinct cavity with visible dentin

Table 2. Criteria for CLSM depth measurements according to Moazin.[10]

Score	Description	CLSM depth
0	No lesion	<=30 µm
1	Small lesions	>30-100 µm
2	Medium lesions	>100-500 µm
3	Large lesions	>500 µm

Table 3. ICCs values for intra- and inter-examiner reproducibility for all methods for both groups.

Method	Group A		Group B	
	Intra-Examiner	Inter-Examiner	Intra-Examiner	Inter-Examiner
ICDAS	0.87	0.81	0.90	0.85
LF	0.98	0.94	0.89	0.88
MID	0.45	0.45	0.83	0.77
QLF	0.92	0.87	0.93	0.90
PCDS	0.79	0.82	0.85	0.88

Table 4. Specificity (Sp), sensitivity (Se), accuracy (Ac) and area under the ROC curve (Az) for all methods for both groups considering overall lesions*.

Method	Group A			
	Sp	Se	Ac	Az
ICDAS	0.70[a]	0.20[a]	0.35[a]	0.56[a]
LF	0.84[a,b]	0.05[b]	0.29[a]	0.57[a]
MID	0.87[b]	0.05[b]	0.30[a]	0.55[a]
QLF	0.29[c]	0.61[c]	0.51[b]	0.57[a]
PCDS	0.25[c]	0.71[c]	0.57[b]	0.57[a]
Method	Group B			
	Sp	Se	Ac	Az
ICDAS	0.73[a]	0.71[a]	0.72[a]	0.75[a]
LF	0.83[a,b]	0.70[a]	0.74[a]	0.82[a]
MID	0.92[b]	0.37[b]	0.54[b]	0.65[b]
QLF	0.53[c]	0.84[c]	0.74[a]	0.82[a]
PCDS	0.57[c]	0.79[c]	0.72[a]	0.73[a]

Significant differences are represented by different superscript letters, considering the same column (Bootstrap methodology, $p<0.05$)

*Overall lesions: caries if CLSM>0 and if any methods scores >0

Table 5. Sensitivity values for all methods at D_1, D_2 and D_3 thresholds for both groups.

Method	Group A			Group B		
	D_1	D_2	D_3	D_1	D_2	D_3
ICDAS	0.30^{a}	0.19^{a}	0.18^{a}	0.38^{a}	0.65^{a}	$0.83^{a,d}$
LF	0.00^{a}	0.04^{a}	0.06^{a}	0.38^{a}	0.69^{a}	0.77^{a}
MID	0.00^{a}	0.07^{a}	0.05^{a}	0.00^{b}	0.25^{b}	0.55^{b}
QLF	0.66^{b}	0.64^{b}	0.58^{b}	0.51^{a}	0.76^{a}	$0.97^{c,d}$
PCDS	0.49^{c}	0.72^{b}	0.74^{b}	0.50^{a}	0.72^{a}	0.91^{d}

D_1 Threshold: caries if CLSM=1
D_2 Threshold: caries if CLSM=2
D_3 Threshold: caries if CLSM=3
Significant differences are represented by different superscript letters, considering the same column (Bootstrap methodology, $p<0.05$)

Table 6. Spearman correlation coefficients between all methods and CLSM depth analysis for both groups.

Method	Group A	Group B
ICDAS	-0.11	0.55*
LF	-0.05	0.54*
MID	-0.12	0.44*
QLF	-0.09	0.65*
PCDS	-0.01	0.51*

*$p<0.05$, Bootstrap test.

Table 7. Spearman correlation coefficients between the accuracy of the gap size and all methods for both groups.

Method	Group A	Group B
ICDAS	0.45*	0.58*
LF	0.26*	0.45*
MID	0.17	0.52*
QLF	0.36*	0.65*
PCDS	0.33*	0.62*

*p<0.05, Bootstrap test.

Table 8. Cross-tabulation for the accuracy of the gap size and the CLSM depth scores for both groups.

Group A Gap Size	CLSM depth				
	0	1	2	3	Total
0	19%	5%	18%	25%	67%
1	7%	1%	7%	8%	23%
2	4%	1%	3%	2%	10%
Total	30%	7%	28%	35%	100%
Group B Gap Size	**CLSM depth**				
	0	1	2	3	Total
0	27%	5%	16%	9%	57%
1	4%	2%	10%	9%	25%
2	0%	0%	2%	16%	18%
Total	31%	7%	28%	34%	100%

CONSIDERAÇÕES FINAIS

Considerações Finais

O diagnóstico da doença cárie é um processo amplo e abrangente, envolvendo não somente na detecção das lesões de cárie, mas também na avaliação de outros fatores, como a determinação do risco e da atividade da doença e na coleta de informações do paciente, como os hábitos de higiene oral, dieta e uso de fluoretos. Assim, esse conjunto de informações será de extrema importância para a decisão de tratamento de cada paciente, favorecendo um melhor prognóstico no controle da doença.

A detecção de lesões de cárie em superfícies oclusais é extremamente difícil e complexa para o clínico. Esse fato é atribuído às mudanças nas características clínicas e na progressão dessas lesões devido ao advento dos fluoretos. Assim, novos métodos vêm sendo desenvolvidos e avaliados como auxiliares aos exames convencionais na detecção e na quantificação de lesões de cárie.

Diversos estudos têm mostrado bom desempenho de métodos baseados na captação da fluorescência induzida por luz na detecção de lesões cariosas primária (Lussi et al.[46], 1999; Ando et al.[3], 2001; Lussi et al.[48], 2001; Lussi, Hellwig[45], 2006; Lussi et al.[44], 2006; Thoms[68], 2006; Krause et al.[37], 2007; Braun et al.[15], 2008; Huth et al.[30], 2008; Rodrigues et al.[62], 2008; Diniz et al.[18], 2009; Kano-Wilson et al.[33], 2009; De Benedetto et al.[17], 2010) e secundária (Boston[10], 2003; Ando et al.[2], 2004; Bamzahim et

al.[7], 2005; Braga et al.[11], 2010) e a importância da limpeza da superfície dentária para a realização de um correto exame. Estudos têm mostrado que a presença de placa bacteriana e remanescentes de pastas e/ou pó profiláticos nos sulcos e fissuras podem influenciar negativamente o desempenho desses métodos (Lussi et al.[46], 1999; Mendes et al.[51], 2004; Anttonen et al.[6], 2005; Lussi, Reich[49], 2005).

Este trabalho está sendo apresentando em forma de quatro capítulos. No primeiro capítulo foi avaliada a influência de dois métodos de profilaxia profissional nas medidas de fluorescência e no desempenho de métodos baseados na captação da fluorescência induzida por luz na detecção de lesões de cárie oclusal em dentes permanentes. Os resultados obtidos demonstraram excelente reprodutibilidade dos métodos de detecção de cárie avaliados. Além disso, observou-se que tanto o jato de bicarbonato de sódio como a pasta profilática podem influenciar significativamente as medidas de fluorescência do DIAGNOdent, do DIAGNOdent *pen* e da câmera VistaProof. O grupo da pasta profilática apresentou maiores medidas de fluorescência quando comparado ao grupo do jato de bicarbonato. É importante ressaltar que a lavagem cuidadosa após a profilaxia com pasta profilática foi extramemente importante para remover os remanescentes da pasta e melhorar o desempenho dos métodos na detecção de lesões de cárie oclusais.

O desempenho dos métodos baseados na captação da fluorescência induzida por luz está diretamente relacionado com os pontos de corte empregados na clínica odontológica. O grande desafio do cirurgião dentista é decidir pelo ponto de corte mais adequado para cada método e interpretar os resultados obtidos corretamente. O problema é que tanto os fabricantes como a literatura propoem diferentes pontos de corte, que diferem em algumas unidades, fato este que dificulta o emprego dos métodos na prática clínica. Outro ponto crucial está relacionado com a decisão de tratamento, uma vez que outros fatores devem ser considerados, como a atividade de cárie, o risco à cárie do paciente, o uso de fluoretos e a dieta.

No segundo capítulo foram determinados pontos de corte clínicos ideiais para o DIAGNOdent, o DIAGNOdent *pen* e a VistaProof. Além disso, avaliou-se a validade de métodos convencionais e de métodos baseados na captação da fluorescência induzida por luz na detecção de lesões de cárie oclusal in vivo com total validação histológica da amostra. Neste estudo, observamos que o critério visual ICDAS, o DIAGNOdent e o DIAGNOdent *pen* apresentaram boa validade na detecção de lesões de cárie oclusal. Sugerimos que esses métodos oferecem resultados promissores na prática clínica. Os resultados confirmam também que o exame radiográfico interproximal apresenta baixa validade em detectar as lesões de cárie oclusais e deve ser utilizado com cautela. É importante enfatizar que o

cirurgião dentista deve ter em mente que as medidas de fluorescência não devem ser interpretadas como medidas únicas, mas sim como intervalo de valores. Sugerimos que esses métodos auxiliares sejam sempre utilizados com cautela e como uma segunda opinião na detecção de lesões de cárie.

Normalmente, o exame clínico da avaliação da atividade de cárie baseia-se na utilização de métodos convencionais, como a inspeção visual e o exame táctil, para determinar subjetivamente as características clínicas indicativas de atividade. Recentemente, o critério LAA foi desenvolvido para ser empregado associado ao critério visual ICDAS para avaliar a atividade de lesões de cárie, apresentando bons resultados (Ekstrand et al.[20], 2007; Braga et al.[12], 2009).

No terceiro capítulo, o critério ICDAS-LAA e o critério clínico visual/táctil foram comparados in vivo na avaliação da atividade de lesões de cárie oclusal em dentes decíduos. Posteriormente, esses resultados in vivo foram comparados com os resultados in vitro obtidos pelo Carivis, um novo método baseado em luminescência. Ambos critérios também foram comparados com esse novo método para determinar o status da atividade de lesões de cárie reexaminadas após 2 meses de acompanhamento clínico. Os resultados mostraram que ambos os critérios clínicos apresentaram uma alta correlação quando empregados in vivo. Além disso, o método de luminescência apresentou capacidade em avaliar a atividade de lesões de

cárie in vitro. Sugerimos que novos estudos sejam realizados para avaliar o desempenho do método de luminescência na avaliação da atividade de lesões de cárie e determinar o critério clínico mais acurado para ser empregado na clínica odontológica.

É importante enfatizar que os métodos devem ser indicados tanto para a detecção de lesões de cárie primária como também secundária, que é o principal motivo das substituições das restaurações dentárias, sendo o grande desafio a diferenciação entre as pigmentações e os defeitos marginais não cariosos dos cariosos.

No quarto capítulo foi avaliado o desempenho de métodos na detecção de lesões naturais de cárie ao redor de restaurações de amálgama e de resina composta em dentes permanentes e determinou a relação entre a presença da lesão de cárie secundária e a presença/ausência de diferentes tamanhos de defeitos marginais. Foram utilizados o ICDAS, o DIAGNOdent, o Midwest Caries I.D., o QLF e o PCDS. Os resultados mostraram que o critério visual ICDAS e o DIAGNOdent apresentaram bom desempenho na detecção de lesões de cárie ao redor de restaurações de resina composta, e que o QLF e o PCDS na detecção de lesões ao redor de restaurações de amálgama e de resina composta. Pôde-se concluir também que o tamanho do defeito marginal foi irrelevante na determinação da presença de lesões de cárie ao redor de restaurações.

Diante do presente trabalho, observou-se que os métodos descritos para detecção e avaliação da atividade de lesões de cárie parecem contribuir de forma positiva no processo de diagnóstico. No entanto, devem ser considerados como auxiliares aos métodos convencionais nesse processo.

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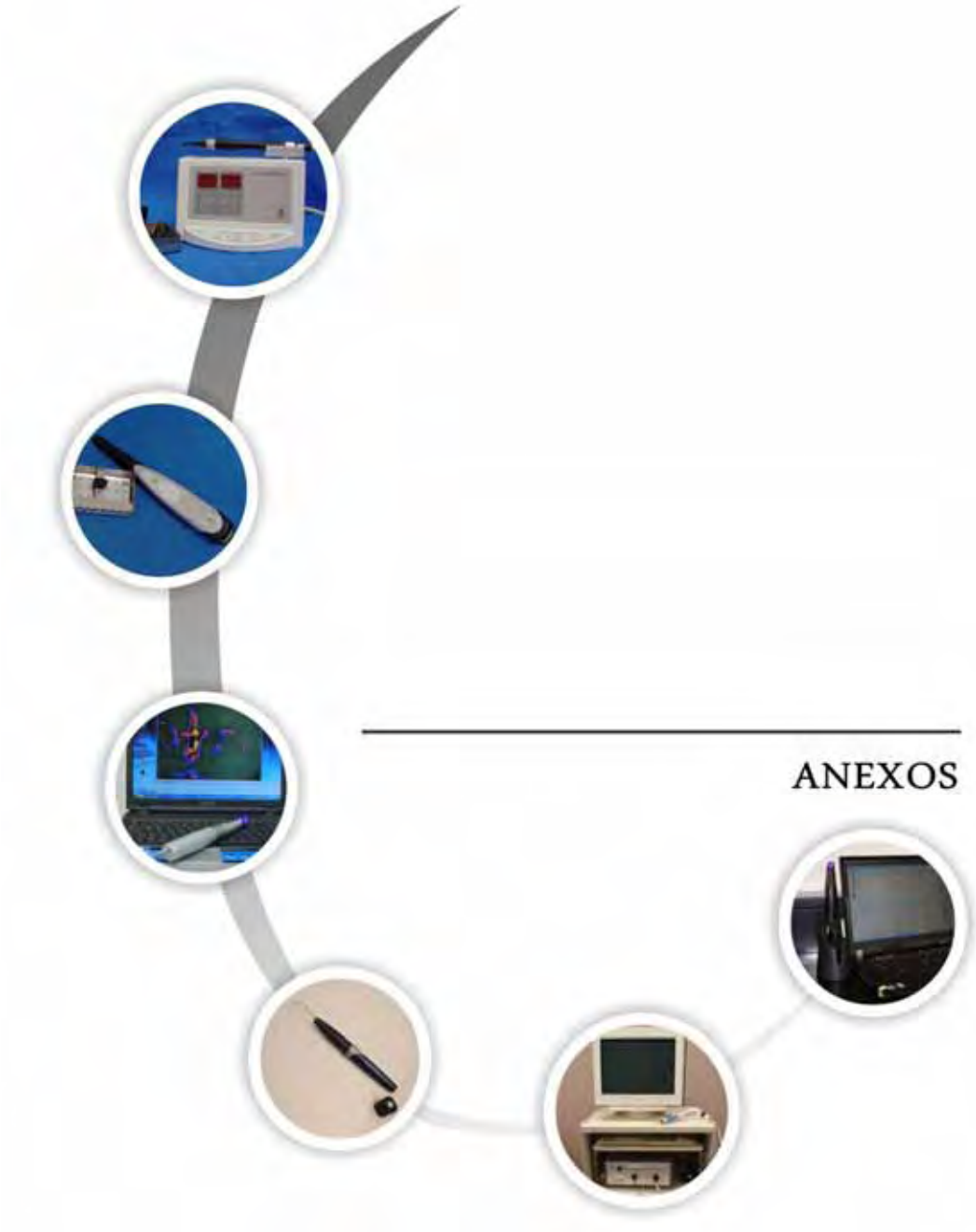


ANEXOS

UNIVERSIDADE ESTADUAL PAULISTA " JÚLIO DE MESQUITA FILHO"

FACULDADE DE ODONTOLOGIA DE ARARAQUARA

Comitê de Ética em Pesquisa

Certificado

Certificamos que o projeto de pesquisa intitulado *"AVALIAÇÃO DO DESEMPENHO DOS MÉTODOS BASEADOS EM INDUÇÃO DE FLUORESCÊNCIA NA DETECÇÃO DE LESÕES DE CÁRIE OCLUSAL. ESTUDOS IN VITRO E IN VIVO"*, sob o protocolo nº 04/08, de responsabilidade do Pesquisador (a) *RITA DE CÁSSIA LOIOLA CORDEIRO,* 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–FOAr, com validade de 02 (dois) anos, quando será avaliado o relatório final da pesquisa.

Certify that the research project titled *"PERFORMANCE OF FLUORESCENCE-BASED METHODS IN DETECTING OCCLUSAL CARIES LESIONS. IN VITRO AND IN VIVO STUDIES"*, protocol number 04/08, under Dr. *RITA DE CÁSSIA LOIOLA CORDEIRO,* responsability, is under the terms of Conselho Nacional de Saúde/MS resolution # 196/96, published on May 10, 1996. This research has been approved by Research Ethic Committee, FOAr-UNESP. Approval is granted for 02 (two) years when the final review of this study will occur.

Araraquara, 30 de maio de 2008.

Profª Drª Mirian Aparecida Onofre
Coordenadora

UNIVERSIDADE ESTADUAL PAULISTA " JÚLIO DE MESQUITA FILHO"

FACULDADE DE ODONTOLOGIA DE ARARAQUARA

Comitê de Ética em Pesquisa

Certificado

Certificamos que o projeto de pesquisa intitulado ***"AVALIAÇÃO DO ICDAS-II E DE MÉTODOS BASEADOS EM INDUÇÃO DE FLUORESCÊNCIA PARA DETECÇÃO DE LESÕES DE CÁRIE PRIMÁRIA E SECUNDÁRIA"***, sob o protocolo nº **48/08**, de responsabilidade do Pesquisador (a) *RITA DE CÁSSIA LOIOLA CORDEIRO*, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pela CONEP, conforme Parecer nº 184/2009, com validade de 02 (dois) anos, quando será avaliado o relatório final da pesquisa.

Certify that the research project titled ***"EVALUATION OF ICDAS-II AND FLUORESCENCE-BASED METHODS FOR PRIMARY AND SECONDARY CARIES DETECTION"***, protocol number **48/08**, under Dr *RITA DE CÁSSIA LOIOLA CORDEIRO* responsability, is under the terms of Conselho Nacional de Saúde/MS resolution # 196/96, published on May 10, 1996. This research has been aproved by CONEP Parecer nº 184/2009. Approval is granted for 02 (two) years when the final review of this study will occur.

Araraquara, 18 de maio de 2009.

Profª Drª Mirian Aparecida Onofre
Coordenadora

Anexo C

UNESP UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE ARARAQUARA
FACULDADE DE ODONTOLOGIA

Termo de Consentimento Livre e Esclarecido

Por este instrumento particular, declaro para os devidos fins éticos e legais, que eu, ..., portador(a) do RG nº ....., residente à ....., nº....., na cidade de ....., Estado de ....., ... (grau de parentesco) e responsável pelo(a) menor ....., ... anos, prontuário ..... concordo voluntariamente com sua participação na pesquisa **"Avaliação do desempenho dos métodos baseados em indução de fluorescência na detecção de lesões de cárie oclusal. Estudos in vitro e in vivo"**, tendo como pesquisadora responsável Rita de Cássia Loiola Cordeiro, professora da Disciplina de Odontopediatria da Faculdade de Odontologia de Araraquara - UNESP. E declaro que tomei ciência e que fui esclarecido(a) de maneira a não restarem quaisquer dúvidas sobre sua participação no estudo, de acordo com os termos abaixo relacionados:

1) O objetivo deste estudo é avaliar se três aparelhos de laser, a radiografia e o método visual são bons para detectar a lesão de cárie em dentes permanentes.
2) Primeiramente será realizado um exame dos dentes indicados para extração.
3) Após limpeza do dente, será realizado um exame visual do dente e exame com três aparelhos de laser que emitem luz vermelha ou azul no dente. Estes métodos são não-invasivos e não oferecem perigo, pois a luz emitida encontra-se na faixa visível a olho nu.
4) Em seguida, o dente será extraído sob anestesia local na Clinica de Cirurgia desta Faculdade, tendo como cirurgião-dentista responsável o Prof. Dr....., uma vez que não há possibilidade de receber tratamento conservador.
5) Estou ciente que o dente será extraído por necessidade independente da pesquisa.
6) Estou ciente também que o dente extraído será doado ao pesquisador.

Anexo C

7) Estou consciente de que o participante da pesquisa não terá benefício direto com este estudo mas estou disposto a autorizar sua participação para que os resultados encontrados possam ajudar outras pessoas.
8) Estou consciente também que o exame com os aparelhos a laser não causam desconforto ou dor, nem prejuízo à saúde do menor sob minha responsabilidade.
9) Estou ciente que os riscos deste estudo são os mesmos de quando se tira um dente e todos os cuidados serão tomados para evitá-los, utilizando instrumentais esterilizados e protegendo os pacientes com avental de chumbo para a realização das radiografias.
10) Não será oferecido nenhum tipo de pagamento de gastos, assim como não será efetuado nenhum tipo de pagamento pela participação.
11) Tenho total liberdade para recusar ou cancelar a participação do menor, sem nenhuma implicação ao atendimento que receberá nesta Faculdade.
12) O pesquisador responsável garante sigilo quanto aos dados confidenciais envolvidos e a identidade do participante.
13) Tenho total liberdade para solicitar maiores esclarecimentos antes e durante o desenvolvimento da pesquisa e consultar o Comitê de Ética em Pesquisa para qualquer informação sobre o projeto que autoriza participação do mesmo.
14) Autorizo, para devidos fins, o uso, a divulgação e publicação em revistas científicas os dados obtidos nesta pesquisa.
15) Permito que sejam realizadas fotografias dos dentes incluídos na pesquisa, sem que a identidade do participante seja revelada.

Desta forma, confirmo que recebi de maneira clara, todas as informações necessárias ao meu consentimento. Assim, informo que o menor pelo qual sou responsável irá participar desta pesquisa por livre e espontânea vontade.

Araraquara, ... de .....de 2000.....

_____ _____

Assinatura do responsável

Assinatura do pesquisador responsável

Telefones para co

Secretaria do Comitê de Ética em Pesquisa: (016) 3301-6432/3301-6434

Pesquisador responsável: (016) 3301-6331

Anexo D

UNESP UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE ARARAQUARA
FACULDADE DE ODONTOLOGIA

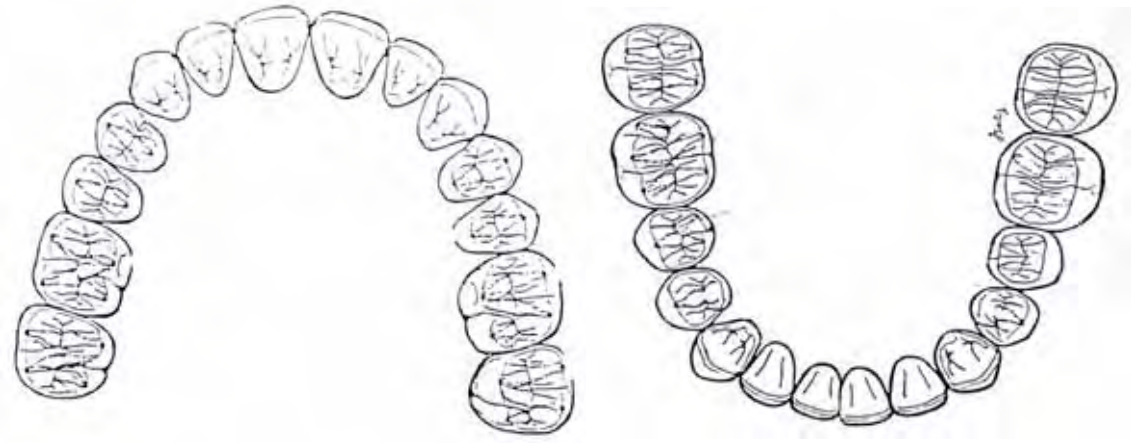
FICHA CLÍNICA

Paciente: ..... **Número:** .....

Idade: ..... **Data do exame:** .....

Dente	Rx	ICDAS	DD		DDPen		VistaProof	
			Valor Zero	Peak	Valor Zero	Peak	Valor	Cor
Sítio 1								
Sítio 2								
Sítio 3								
Sítio 4								

IDENTIFICAÇÃO DOS SÍTIOS



Anexo E

UNESP UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE ARARAQUARA
FACULDADE DE ODONTOLOGIA

Termo de Consentimento Livre e Esclarecido

Por este instrumento particular, declaro para os devidos fins éticos e legais, que eu, ..., ... anos, residente à ....., nº....., na cidade de ....., Estado de ..... fui informado e concordo voluntariamente com minha participação na pesquisa **"Avaliação do ICDAS-II e de métodos baseados em indução de fluorescência para detecção de lesões de cárie primária e secundária"**, tendo como pesquisadora responsável Rita de Cássia Loiola Cordeiro, professora da Disciplina de Odontopediatria da Faculdade de Odontologia de Araraquara - UNESP. E declaro que tomei ciência e que fui esclarecido(a) de maneira a não restarem quaisquer dúvidas sobre minha participação no estudo, de acordo com os termos abaixo relacionados.

Fui informado que:

1) O objetivo deste estudo é avaliar se o método visual e um aparelho a laser são bons para detectar a lesão de cárie em dentes de leite.
2) Serão examinados somente os dentes do fundo que vão "cair" em um período máximo de seis meses.
3) Após limpeza do dente, será realizado um exame visual. Fui esclarecido que este método não oferece perigo ou risco.
4) Em seguida, responderei um questionário com perguntas relacionadas à escovação dos dentes, uso de flúor e cuidados com a minha saúde bucal.
5) Fui informado e estou ciente que o dente irá "cair" sozinho ou será extraído por necessidade independente da pesquisa.
6) Quando o dente "cair sozinho" deverei encaminhá-lo ao pesquisador;
7) Os dentes serão enviados para o Laboratório de Pesquisas em Detecção e Manejo Precoce da Lesão de Cárie para uma Faculdade nos Estados Unidos (*Oral Health Research Institute* - Faculdade de Odontologia da Universidade de Indiana), para realização das análises.

Anexo E

8) A doação do(s) dente(s) será voluntária não sendo oferecido nenhum tipo de pagamento de gastos, assim como não será efetuado nenhum tipo de pagamento pela minha participação, mas caso necessário serei ressarcido das despesas extras que tiver decorrentes da participação na pesquisa.
9) Tenho total liberdade em não participar da pesquisa, sem nenhum prejuízo ao atendimento que estou recebendo na Clínica Infantil desta Faculdade.
10) Estou ciente que esta pesquisa não vai interferir no tratamento que foi planejado para os meus dentes, e que este tratamento, inclusive a assistência integral necessária por qualquer problema decorrido, é de total responsabilidade da Clínica de Odontopediatria.
11) Minha identidade será mantida em sigilo e que tenho total liberdade para solicitar maiores esclarecimentos antes e durante o desenvolvimento da pesquisa e consultar o Comitê de Ética em Pesquisa para qualquer informação sobre o projeto.
12) Estou ciente que não terei benefício direto com este estudo, mas estou disposto a autorizar minha participação para que os resultados encontrados possam ajudar outras pessoas.

Portanto,

1. Autorizo, para devidos fins, o uso, a divulgação e publicação em revistas científicas os dados obtidos nesta pesquisa.
2. Permito que sejam realizadas fotografias dos dentes incluídos na pesquisa, desde que minha identidade não seja revelada
3. Concordo em doar o(s) dente(s) ..... para a pesquisa.

Desta forma, confirmo que recebi de maneira clara, todas as informações necessárias ao meu consentimento. Assim, informo que irei participar desta pesquisa por livre e espontânea vontade.

Araraquara, ... de .....de 200.....

Assinatura do menor	Assinatura do pesquisador responsável

Anexo E

Eu, ..., portador(a) do RG nº ....., residente à ....., nº....., na cidade de ....., Estado de ....., estou ciente das informações acima e autorizo o menor ..... a participar da pesquisa.

_____

Assinatura do responsável

Telefones para contato

Secretaria do Comitê de Ética em Pesquisa: (016) 3301-6432/3301-6434

Pesquisador responsável: (016) 3301-6331

Anexo F

UNESP UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE ARARAQUARA
FACULDADE DE ODONTOLOGIA

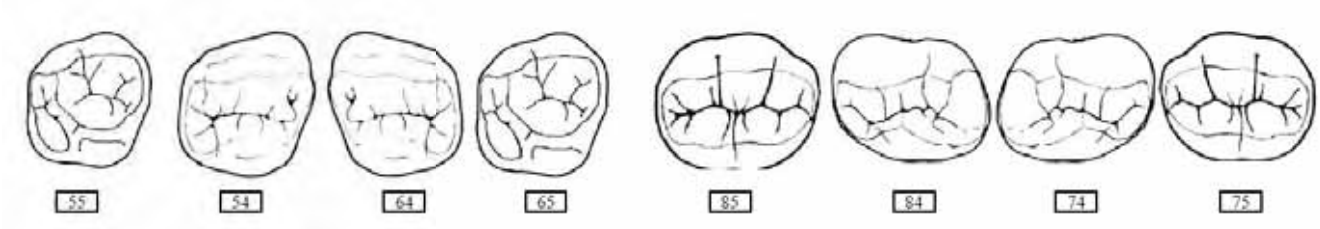
FICHA CLÍNICA

Paciente: ..... **Número:** .....

Idade: ..... **Data do exame:** .....

Dente	ICDAS	*Lesion Activity Assessment* (LAA)				Visual/ táctil
		Aparência clínica	Estagnação de placa	Textura da superfície	Total	
54						
55						
64						
65						
74						
75						
84						
85						

)A



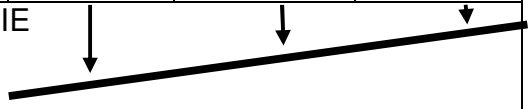
Anexo G

FORMULÁRIO DE AVALIAÇÃO DO RISCO À CÁRIE (CAMBRA)

Nome do paciente: .....
Idade: ..... Data: .....

Indicadores da doença cárie	***SIM (circular)***	***SIM (circular)***	***SIM (circular)***
Cavidades clinicamente visíveis ou radiolucidez em dentina visível radiograficamente	**SIM**		
Lesões de cárie proximais visíveis radiograficamente (não em dentina)	**SIM**		
Manchas brancas em superfícies lisas	**SIM**		
Restaurações nos últimos 3 anos	**SIM**		
Fatores de risco			
Placa visível na superfície dentária		**SIM**	
Lanches freqüentes (>3x ao dia entre as refeições)		**SIM**	
Fóssulas e fissuras profundas		**SIM**	
Fluxo salivar inadequado por observação		**SIM**	
Fatores de redução da saliva (medicamentos/radiação/sistêmicos)		**SIM**	
Aparelhos ortodônticos		**SIM**	
Fatores de proteção			
Água fluoretada (residência, escola)			**SIM**
Dentifrício fluoretado pelo menos 1x/dia			**SIM**
Dentifrício fluoretado pelo menos 2x/dia			**SIM**
Bochecho com flúor (0,05% NaF) 1x/dia			**SIM**
Dentifrício fluoretado contendo 5000 ppm F 1x/dia			**SIM**
Verniz fluoretado nos últimos 6 meses			**SIM**
ATF profissional nos últimos 6 meses			**SIM**
Prescrição de cloredixina/ 1x/semana nos últimos 6 meses			**SIM**
Chiclete de xilitol 4x/dia nos últimos 6 meses			**SIM**
Pasta de cálcio e fosfato durante os últimos 6 meses			**SIM**

VISUALIZAÇÃO DO BALANÇO DE CÁRIE
(indicadores circulados/fatores)



RISCO À CÁRIE: ( ) Alto ( ) Moderado ( ) Baixo

Assinatura do responsável: .....

Assinatura do pesquisador: .....

Araraquara, 28 setembro de 2010

MICHELE BAFFI DINIZ