

UNIVERSIDADE FEDERAL DE PERNAMBUCO
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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
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ÁREA DE CONCENTRAÇÃO EM CLÍNICA INTEGRADA

**AVALIAÇÃO DE TÉCNICAS DIAGNÓSTICAS DE LESÃO DE CÁRIE PROXIMAL:
RAIO X, TRANSILUMINAÇÃO COM RADIAÇÃO INFRAVERMELHO PRÓXIMO
E TOMOGRAFIA POR COERÊNCIA ÓPTICA**

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ANA MARLY ARAÚJO MAIA

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RAIO X, TRANSILUMINAÇÃO COM RADIAÇÃO INFRAVERMELHO PRÓXIMO
E TOMOGRAFIA POR COERÊNCIA ÓPTICA**

Dissertação apresentada à Coordenação do Curso de Mestrado em Odontologia, com área de concentração em Clínica Integrada, como requisito parcial para obtenção do grau de Mestre em Odontologia.

Orientador: Prof. Dr. Anderson S. L. Gomes

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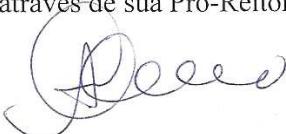
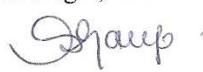
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SECRETARIA

Oziclere de Araújo Sena

Ata da 75ª Defesa de Dissertação do Curso de Mestrado em Odontologia com área de Concentração em Clínica Integrada do Centro de Ciências da Saúde da Universidade Federal de Pernambuco. Recife, 29 de janeiro de 2009.

Às 9:00(nove horas) do dia 29 (vinte e nove) do mês de janeiro do ano de dois mil e nove, reuniram-se no Auditório Ageu de Aquino Sales do Curso de Odontologia da Universidade Federal de Pernambuco, os membros da Banca Examinadora, composta pelos professores: Profa.Dra. ALESSANDRA DE ALBUQUERQUE TAVARES CARVALHO , atuando como presidente, Prof.Dr. ALEXANDRE LOPES DO NASCIMENTO, da Universidade de Pernambuco, atuando como primeiro examinador. Prof. Dr. CLAUDIO HELIOMAR VICENTE DA SILVA , atuando como segundo examinador, para julgar o trabalho intitulado **“AVALIAÇÃO DE TECNICAS DIAGNÓSTICAS DE LESÃO DE CÁRIE INTERPROXIMAL: RAOX, TRANSILUMINAÇÃO COM RADIAÇÃO INFRAVERMELHO PRÓXIMO E TOMOGRAFIA POR COERENCIA ÓPTICA”** da CD. **ANA MARLY ARAUJO MAIA**, candidata ao Grau de Mestre em Odontologia, na Área de Concentração em CLINICA INTEGRADA, sob orientação do Professor Dr. ANDERSON STEVENS LEONIDAS GOMES. Professora Dra. ALESSANDRA DE ALBUQUERQUE TAVARES CARVALHO, membro do Programa de Pós Graduação em Odontologia abriu os trabalhos convidando os senhores membros para compor a Banca Examinadora, foram entregues aos presentes cópias do Regimento Interno do Curso de Mestrado em Odontologia, que trata dos critérios de avaliação para julgamento da Dissertação de Mestrado. A presidente da mesa após tomar posse conferiu os membros, seguindo convidou a mestrandona. CD. **ANA MARLY ARAUJO MAIA** , para expor sobre o aludido tema, tendo sido concedido trinta minutos. A candidata expôs o trabalho e em seguida colocou-se a disposição dos Examinadores para argüição. Após o término da argüição os Examinadores reuniram-se em secreto para deliberações formais. Ao término da discussão, atribuíram a candidata os seguintes conceitos: Prof.Dr. ALEXANDRE BATISTA LOPES DO NASCIMENTO (**APROVADA**), Prof. Dr. CLAUDIO HELIOMAR VICENTE DA SILVA , (**APROVADA**), Profa.Dra. ALESSANDRA DE ALBUQUERQUE TAVARES CARVALHO (**APROVADA**), a candidata recebeu três conceitos (**APROVADA**) é considerada (**APROVADA COM DISTINÇÃO**), devendo a candidata acatar as sugestões da Banca Examinadora de acordo com o Regimento Interno do Curso, face a aprovação, fica a candidata, apta a receber o Grau de Mestre em Odontologia, cabendo a Universidade Federal de Pernambuco através de sua Pró-Reitoria para



Assuntos de Pesquisa e Pós-Graduação, tomar as providências cabíveis. Nada mais havendo a tratar, a Presidente da Banca Examinadora encerrou a sessão e para constar eu, Oziclere Sena de Araújo, lavrei a presente Ata que vai por mim assinada, pelos demais componentes da Banca Examinadora e pelo recém formado mestre pela UFPE, . **ANA MARLY ARAUJO MAIA.**

Ana Marly Araujo Maia

Recife, 29 de janeiro de 2009.

Alessandra de Albuquerque Tavares Carvalho
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Oziclere Sena
Senhora Oziclere

DEDICATÓRIA

... a Deus

pela dom da vida. Por ter me abençoado com coragem, ânimo e sabedoria para viver as novas experiências. *“Toda honra e toda glória, é dEle a vitória que alcançou em minha vida”*

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LISTA DE ABREVIATURAS E SIGLAS

ANSI - American National Standards Institute
C - Contrast
CCD – Charge-Coupled device
D – Dentin
DIFOTI – Digital Imaging Fiber-Optic Trans-Illumination
E - Enamel layer
EDJ - Enamel-dentin junction
Ex - Examiner
FDA – Food and Drug Administration
FN – False negative/Falso negativo
FOTI - Fiber-Optic Trans-Illumination
FP – False positive/ Falso positivo
ICC - Intra-class Correlation Coefficient
 I_{\max} - maximum intensity
 I_{\min} - the lowest value.
IR – Índice de refração
Laser – Light Amplification by Stimulated Emission of Radiation
LF – Light Fluorescence
lp/mm – line pairs / milímetros
mW - miliwatts
NIR – Near Infra Red
nm – nanômetro
NV - negativo verdadeiro
PV - positivo verdadeiro
QLF – Quantitative Light Fluorescence
ROC - Receive Operator Characteristic Curve
S – Sensitivity /Sensibilidade
S – Specificity/ Especificidade
SD-OCT - spectral domain/ domínio espectral
SLD - Super Luminescent Diodes
TCO (OCT) – Tomografia por Coerência Óptica

TD-OCT - time domain/ domínio de tempo

TI – Transillumination

TN - True-negative

TP - True-positive

λ - Lambda

μm - micrômetro

APRESENTAÇÃO

Esta dissertação foi organizada em forma de artigos científicos para serem enviados a revistas internacionais, especializadas na área de interface entre a Física Biomédica e Odontologia, precedido por uma introdução ao tema e revisão bibliográfica. A revisão de literatura em bancos de dados eletrônicos internacionais pesquisou a complexidade dos avançados métodos de diagnóstico de cárie precoce, elucidando técnicas que avaliam propriedades ópticas da interação laser-tecido, como a Transiluminação (TI, sigla em inglês) com infravermelho próximo (NIR, sigla em inglês), e Tomografia por coerência óptica (TCO). De acordo com os objetivos de avaliar as técnicas de forma integral, esta dissertação resultou em três artigos que apresentam inicialmente a montagem do sistema ótico e avaliação da resolução ótica, bem como a sensibilidade e especificidade no diagnóstico da cárie precoce. O primeiro artigo, intitulado de “**Evaluation of Near-Infrared Transillumination System for Early Caries Detection**”, a ser encaminhado ao Journal of Biomedical Optics, consiste em uma análise da resolução espacial do sistema de Transiluminação com Infravermelho Próximo tornando possível afirmar as possibilidades e limitações da técnica, quanto à menor visualização de uma lesão de cárie, elucidando interferências quanto a propagação da luz no tecido em diferentes espessuras de esmalte. Este teste inicial foi essencial para definir a metodologia utilizada. Os resultados obtidos mostraram uma visualização da cárie quantitativamente e provaram que a resolução do sistema não é um fator limitante. O segundo artigo intitulado, “**Evaluation of sensibility and specificity of NIR TI and X Ray images of noncavitated caries lesions**”, que será encaminhado a Photomedicine and Laser Surgery, proporcionou continuidade à avaliação da técnica. Um total de 28 imagens, referente a dentes sadios e com manchas brancas iniciais nas superfícies proximais, foram realizadas pela técnica de transiluminação com infravermelho próximo e pelo raio x convencional digitalizado. As imagens foram avaliadas por especialistas em Radiologia Odontológica, quanto à sensibilidade e especificidade, cujo resultado comprovou a eficácia da Transiluminação quando comparada ao raio x, comumente utilizado na prática clínica diária. Como resultado, ficou demonstrado que a sensibilidade da transiluminação é duas vezes melhor que o raio x enquanto que a especificidade foi semelhante nos dois casos. O terceiro artigo, intitulado “**Characterization of enamel**

in deciduous teeth by Optical Coherence Tomography: in vitro comparison between 850nm and 1280nm", que será enviado para publicação na International Journal of Paediatric Dentistry, avaliou a aplicação da Tomografia por Coerência Óptica em dentes deciduos comparada a cortes histológicos e microscopia óptica como padrão ouro. Os resultados demonstraram a eficácia da TCO em visualizar com qualidade as alterações ópticas ocorridas no tecido num processo de desmineralização. Também comprovou que o sistema com comprimento de onda em 1280nm apresenta maior profundidade de penetração em esmalte quando comparado ao de 850nm, devido às diferenças do coeficiente de espalhamento e de absorção.

Em geral, conclui-se que a Transiluminação no infravermelho próximo e a TCO apresentam grande potencial de serem utilizadas na prática clínica proporcionando diagnóstico precoce, e consequentemente, o estabelecimento de métodos profiláticos e preventivos, para reversão ou controle do processo carioso.

Descritores: Diagnóstico cárie precoce, Tomografia por Coerência Óptica, Transiluminação com Infra-vermelho Próximo.

PRESENTATION

This work was organized in the form of scientific papers to be sent to international journals, specializing in the interface between Physics and Biomedical Dentistry, preceded by an introduction to the topic and literature review. The review of literature in electronic databases, international research on the complexity of advanced methods for early diagnosis of caries, clarify techniques that evaluate optical properties of laser-tissue interactions, such as transillumination (TI, acronym in English) with near infrared (NIR, English acronym), and by optical coherence tomography (OCT). According to evaluate the techniques in full, this work resulted in three articles that present the first assembly of the optical system and optical evaluation of the resolution and the sensitivity and specificity in the diagnosis of early caries. The first article, entitled the "**Evaluation of Near-Infrared Transillumination System for Early Caries Detection**", to be sent to the Journal of Biomedical Optics, is an analysis of the spatial resolution of the system of Near Infrared transillumination with making it possible to affirm the possibilities and limitations of the art on display in a minor injury of caries, clarify how the interference of light propagation in tissue in different thicknesses of enamel. This initial test was essential to define the methodology used. The results showed a preview of caries and quantitatively proved that the resolution of the system is not a limiting factor. The second article entitled, "**Evaluation of sensitivity and specificity of NIR TI and x Ray Images of noncavitated caries lesions**," which will be forwarded to Photomedicine and Laser Surgery, has continued the evaluation of the technique. A total of 28 images, for healthy teeth and white patches on surfaces initial proximal, were performed by the technique of transillumination with near infrared and the conventional x ray scanned. The images were evaluated by specialists in Dental Radiology, on the sensitivity and specificity, the result proved the effectiveness of transillumination when compared to x ray, commonly used in daily clinical practice. As a result, it was shown that the sensitivity of transillumination is two times better than the x ray while the specificity was similar in both cases. The third article, entitled "**Characterization of deciduous teeth enamel in teeth by Optical Coherence Tomography: in vitro comparison between 850nm and 1280nm**," which will be sent for publication in the International Journal of Pediatric Dentistry, evaluated by the application of optical coherence

tomography in primary teeth compared the histological sections and optical microscopy as gold standard. The results demonstrated the effectiveness of OCT viewing with optical quality changes occurring in the tissue in a process of demineralization. It also proved that the system with a wavelength of 1280nm presented in greater depth of penetration in enamel when compared to 850nm of the differences of the coefficient of scattering and absorption.

In general, it appears that in the near infrared transillumination and OCT have great potential to be used in clinical practice by providing early diagnosis and, consequently, the establishment of prophylactic and preventive methods to control or reverse the carious process.

Keywords: Early caries diagnosis, Optical Coherence Tomography, Near Infra-red transillumination.

1 INTRODUÇÃO

Indubitavelmente, apesar do desenvolvimento evidente da Odontologia Restauradora, a cárie dentária constitui ainda o interesse principal da saúde oral em todo o mundo. Nas últimas décadas, os esforços foram focalizados na preservação da estrutura dentária, estimulando o uso de medidas preventivas, não invasivas ao invés do tratamento curativo (PALMA DIBB et al., 2000).

O diagnóstico da lesão de cárie pode ser encarado como um desafio na clínica odontológica, visto que o padrão, prevalência e comportamento dessa doença se alteraram com o uso de fluoretos (BENN, 1994; WENZEL et al., 1993). Devido a vários fatores relacionados com a prevalência de cárie na macromorfologia peculiar da superfície lisa proximal dos dentes (CARVALHO, EKSTRAND, THYLSTRUP, 1989), são observadas dificuldades em se detectar clinicamente a lesão de cárie interproximal (ZANARDO, REGO, 2003).

A importância do diagnóstico precoce da cárie dentária vem sendo extremamente enfatizada desde que se tem o conhecimento amplo do processo de remineralização em lesões de cárries incipientes (KIDD, JOYSTON-BECHAL, 1997). Neste contexto, o sucesso do diagnóstico está diretamente relacionado à detecção da doença no estágio reversível, a partir de métodos diagnósticos disponíveis para observar ao máximo o processo carioso presente (PITTS, 1991). Associado a necessidade de detectar-se precocemente para interceptar lesões proximais reversíveis (PEGORARO, FRANCO, 1994), outra grande preocupação na década de 90 foi a monitoração do comportamento destas lesões ao longo do tempo; isto é: a sua progressão, a sua paralisação ou mesmo a sua regressão, conforme medidas de promoção de saúde aplicadas e avaliadas (BARATIERI et al., 2002).

Uma discussão revisando as considerações básicas da interação da luz com o tecido dentário explica muitas questões e demonstra como a luz pode ser usada mais efetivamente para o diagnóstico. Muitas aplicações são baseadas na modificação ou alteração em certas características da luz ao interagir com os tecidos biológicos (MAIA et al., 2008). A interação laser/tecido resulta primariamente em quatro fenômenos: transmissão, espalhamento, reflexão e absorção. E baseada nessas propriedades, o diagnóstico por meio óticos pode fornecer informações importantes da constituição dos tecidos, de maneira rápida, quantitativa e não destrutiva (PARKER, 2007).

Dentre os métodos auxiliares no diagnóstico da cárie, podemos citar a transiluminação por fibra óptica, o exame endoscópico, uso de luz difusa, iluminação ultravioleta, penetração de corantes, penetração de iodo, resistência elétrica, ultrasom, radiografia digitalizada e xeroradiografia (PITTS, 1991). E na última década, várias novas metodologias de detecção da cárie têm sido desenvolvidas. Muitos desses novos desenvolvimentos são por espectroscopia ou tecnologia baseada em imagens óticas e mostram potencial clínico promissor. Na literatura, encontramos pesquisas relatando aplicações de diversos meios para o diagnóstico de cárie, como a *digital imaging fibre-optic trans-illumination* (DIFOTI) (SCHNEIDERMAN et al., 1997), *quantitative light-induced fluorescence* (QLF) (JOSSELIN DE JONG et al, 1995), *laser-induced fluorescence* (STOOKEY et al., 1999), *multi-photon imaging* (GIRKIN, HALL, CREANOR, 2000), *infrared thermography* (KANEKO, MATSUYAMA, NAKASHIMA, 1999), *terahertz imaging* (CRAWLEY et al., 2003), tomografia por coerência óptica (AMAECHI et al., 2001), Espectroscopia Raman (HILL, PETROU, 2000; KO et al., 2005) e transiluminação com infravermelho próximo (JONES et al., 2003; BÜHLER, NGAOTHEPPITAK, FRIED, 2005).

Algumas dessas técnicas encontram-se disponíveis comercialmente, como o DIFOTI, QLF e DIAGNOdent (fluorescência induzida por laser), sendo necessário um treinamento de operadores para visualizar e compreender as técnicas e as imagens fornecidas. Inúmeras pesquisas vêm sendo desenvolvidas explorando-se as vantagens e desvantagens, bem como a sensibilidade e especificidade, para averiguar a ausência de resultados falso positivos e falso negativos.

Nesta dissertação desenvolvida em 3 artigos abordaremos 3 diferentes técnicas diagnósticas de cárie precoce, ressaltando a importância das mesmas, mas sempre em busca de maior eficiência, biocompatibilidade e aplicabilidade. Iniciaremos, num primeiro estudo, com a avaliação da resolução ótica da imagem fornecida pela técnica de transiluminação com infravermelho próximo, em seguida, num segundo trabalho, usamos a mesma técnica e fazemos a avaliação da sensibilidade e especificidade comparada ao raio x digital segundo profissionais especialistas em radiologia odontológica, e no terceiro momento, usamos a técnica de tomografia por coerência óptica para caracterização e quantificação da camada de esmalte em dentes decíduos, bem como diagnóstico precoce de cárie.

2. REVISÃO DA LITERATURA

2.1 Cárie dentária

O esmalte dentário é um tecido acelular altamente mineralizado, no qual cristais microscópicos de fosfato de cálcio compreendem 96% do peso seco. O espaço entre os cristais é ocupado por água e material orgânico (4% por volume), tendo alto conteúdo mineral e matriz acelular mínima (EISENMANN, 1998). Os cristais assemelham-se à hidroxiapatita mineral, são longos e finos, com cerca de 50nm de largura em corte transversal, e mais de 100µm em comprimento no eixo, estando densamente reunidos numa organização repetitiva que forma os prismas do esmalte, e dispostos em ângulo reto em relação à junção amelodentinária, variando de 55º a 100º entre os prismas e a superfície externa do dente. Propriedades físicas do esmalte como a densidade, cor, dureza, entre outras, se aproximam às da hidroxiapatita (FEJERSKOV, KIDD, 2005).

A única região em que os prismas estão perpendiculares em relação à superfície dentária é nas extremidades das cúspides e nas faces proximais, não possuindo um trajeto reto da união amelodentinária até a superfície externa. Portanto, os grupos de prismas possuem uma direção sinuosa, sendo isso conhecido como faixas de Hunter-Schreger (CHEVITARESE, FERNANDEZ, 1991). Devido à mudança na orientação dos prismas, cria-se uma situação de menor transmissão de luz e, portanto, uma maior opacidade do esmalte (CATTARUZZA, 2002).

Embora os cristais de hidroxiapatita sejam transparentes, o esmalte tem um índice de refração no visível que oscila em torno de (IR) de 1,64, e enquanto envolvidos em água, que tem um IR em torno de 1,3 no visível, apresenta aparência

translúcida. Por exemplo, se a água for substituída por ar, então o esmalte vai parecer branco calcário-opaco (FEJERSKOV, KIDD, 2005).

Dentre algumas terminologias utilizadas para a cárie dentária, quanto ao local, pode ser classificada como: lesão de superfície lisa, de fóssulas e fissuras; cárie primária, secundária ou recorrente, e residual que seria o tecido desmineralizado e infectado percebido previamente à inserção do material (decorrente de iatrogenia); e quanto à atividade, classificada como ativa ou inativa (FEJERSKOV, KIDD, 2005).

Numa abordagem mais atual, a atividade da lesão cariosa é bastante questionada, visto que não é um processo estático, sempre haverá um contínuo de mudanças transitórias de lesões ativas para inativas e vice-versa, sendo influenciado por medidas diversas como equilíbrio ecológico do biofilme e o desafio cariogênico, mas principalmente pelos hábitos de higiene oral. Sob condições normais, os fluidos orais são supersaturados em relação à hidroxiapatita e fluorapatita, indicando uma tendência de formação de apatitas em cálculos e remineralização de áreas desmineralizadas em lesões cariosas (FEJERSKOV, KIDD, 2005).

O formato da lesão de mancha branca proximal é determinado pela distribuição dos depósitos microbianos entre a faceta de contato e a margem gengival, resultando em uma lesão com aspecto que lembra o formato de um rim. Na superfície proximal lisa, tipicamente, haverá uma área de faceta interdentária circundada por uma área opaca se estendendo em direção cervical. Na superfície opaca do esmalte, cervical à faceta, inúmeros orifícios irregulares são observados, como depressões dos processos de Tomes mais aprofundados e mais irregulares, e também um número maior de orifícios focais erodidos. O esmalte final exibe distintos padrões de dissolução com espaços intercristalinos ampliados. Os estágios iniciais da lesão cariosa são caracterizados por uma dissolução parcial do tecido, deixando

uma camada superficial bem mineralizada com espessura entre 20 e 50 μm , e na subsuperfície, o corpo da lesão com uma perda mineral de 30 a 50% se estendendo em profundidade no esmalte e na dentina (FEJERSKOV, KIDD, 2005).

Atualmente, a lesão de cárie pode ser caracterizada como crônica, de progressão lenta, raramente autolimitante, e na teoria de formação do processo carioso, reafirma-se a necessidade do acúmulo de bactérias orais sobre a superfície do biofilme dental para que haja destruição cariosa, no entanto, não é causa suficiente para que se estabeleça o processo carioso. A lesão cariosa é a manifestação do estágio do processo num determinado ponto no tempo, mas no processo há conceitos e características da cárie que impedem separá-la como doença e não doença, apresentando-se em vários estágios, num contínuo de ganhos e perdas minerais.

O *iceberg* da cárie dentária, mentalizado por Pitts (2001) conceitua a totalidade do processo carioso, organizando as lesões em estágios de forma crescente, conforme severidade, no qual as lesões iniciais subclínicas estariam submersas na base, e as lesões atingindo o tecido pulpar no topo do *iceberg*. Segundo o *iceberg* observado na Figura 1, os métodos tradicionais de detecção de cárie resultam em vasta quantidade de lesões não detectadas. Há argumentos de que dentre as pequenas lesões, apenas uma porcentagem menor progrediriam para condições mais severas. Num modelo mais atual de análise do processo carioso, Pitts esquematizou o *iceberg* em um retângulo, como observado na Figura 2, que tem interferência do monitoramento da lesão, bem como da avaliação quanto à atividade da lesão.

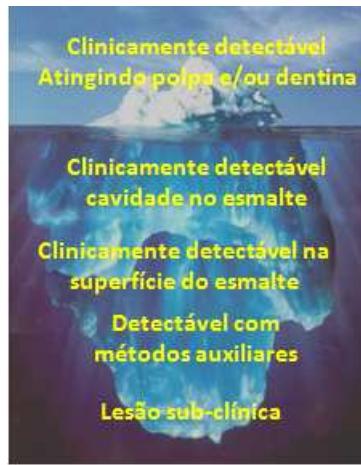


Figura 1: Esquema gráfico proposto por Pitts, representando o processo contínuo da cárie. Adaptado de PITTS, N.B. Modern Concepts od Caries Measurement, 2004.

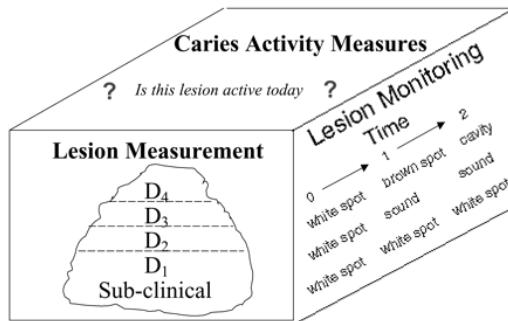


Figura 2: Esquema iceberg inserido num retângulo, apresentando direcionamento da lesão de acordo com grau de severidade, monitoramento da lesão em diferentes momentos, e avaliação da atividade da cárie no topo do processo diagnóstico. Adaptado de PITTS, N.B. Modern Concepts of Caries Measurement, 2004.

O conhecimento do número total de dentes apresentando cárie resumia bem o conceito de diagnóstico de atividade de cárie há algum tempo atrás, ou ainda, o número de novos casos ocorridos em um prazo determinado (incidência). Porém, esta mentalidade estava preocupada apenas com os sinais da doença e não com sua etiologia (WEYNE, 1989).

A doença cárie não se instala a partir do momento em que há lesão visível clinicamente e sim muito antes disto. Assim, a atividade de cárie deve ser considerada alta quando muitos fatores cariogênicos estão presentes e em

condições críticas. Desse modo, novas lesões cariosas podem ser realmente evitadas. Dentro dos novos conceitos de prevenção à doença cárie está o estabelecimento do prognóstico do aparecimento de “novas” lesões, associado ao risco do paciente desenvolver a doença (ADAMS, 1995; NEWBRUN, 1993; WEYNE, 1989).

No modelo atual de determinação social da doença, dentre os determinantes, observa-se que os sociais têm impacto direto no processo saúde-doença, visto que as condições e estilos de vida representam 50% das causas, o meio ambiente 20%, fatores genéticos 20% e a assistência à saúde apenas 10%. Neste contexto, a necessidade de união do conhecimento científico à prática clínica torna-se imprescindível para que técnicas alternativas de tratamento sejam incorporadas, visto que o tratamento restaurador odontológico não influencia na causa principal da doença, condições e estilos de vida. Um questionamento interessante é quanto ao conceito de prevenção como tratamento, pois a melhor medida de controle da doença consiste em como ensinar a remoção da placa bacteriana adequada, aplicação de flúor tópico e discussão sobre a dieta saudável, que podem ser vistas como intervenções, porém, preventivas.

2.2 Diagnóstico de Cárie

Durante nossa revisão da literatura, usaremos a distinção crucial preconizada por Pretty (2006), de que os sistemas apenas detectam a cárie, sendo o diagnóstico o processo de decisão do cirurgião dentista. Estabelecer um correto diagnóstico sempre foi uma preocupação para os profissionais de saúde, já que o sucesso dos procedimentos preventivos e operatórios realizados depende diretamente desta

etapa. Diagnósticos corretos e precisos certamente diferenciam os profissionais, pois aqueles que o realizam tendem a estabelecer um correto tratamento e, consequentemente, solucionam o problema do paciente (BARATIERI et al., 2002).

A identificação da lesão no estágio inicial e a determinação de um tratamento conservativo apropriado permanecem um desafio na prática clínica, não apenas pelas mudanças na morfologia e velocidade da progressão da cárie, mas também pela inexistência de um método capaz de diagnosticar eficazmente tanto o estágio mais precoce do processo carioso, quanto a evolução da mesma, baseada na higidez do elemento dentário (ZANARDO, REGO, 2003), não detectável clinicamente (PALMA DIBB, 2000).

Associado aos métodos diagnósticos disponíveis tem-se o obstáculo da interpretação subjetiva por parte do dentista que com a dificuldade de avaliar a extensão e progressão da lesão, opta por intervenções invasivas injustificadamente. Buscando diagnósticos mais exatos, investigadores têm usado alternativas não-invasivas e técnicas para detectar a quantidade de desmineralização das lesões (HIBST, PAULUS, LUSSI, 2001; LUSSI et al., 1999; FERREIRA-ZANDONÁ et al., 1998). Sendo importante ressaltar, que o diagnóstico e as decisões de tratamento não devem ter base nos sinais clínicos, necessitam de uma análise das condições sociais de vida, bem como de todo o ambiente local da boca do paciente.

Influenciando diretamente na decisão de tratamento, pois depende do cuidadoso diagnóstico da severidade clínica da doença cárie, foi sugerida a presença de cavitação em lesões proximais como o ponto limite para estabelecer o tratamento restaurador, visto que a remoção de placa não é possível em proximais cavitadas (LUNDER, VAN DER FEHR, 1996; PITTS, LONGBOTTOM, 1987).

Angmar-Mänsson e Ten Bosch, em 1987, discutiram métodos óticos para detecção e quantificação de lesões de cárie antes mesmo que a desmineralização se estabelecesse, possibilitando o diagnóstico das lesões em estágios mais iniciais, o que promoveria ao cirurgião-dentista a aplicação de medidas profiláticas, e o monitoramento dessas lesões constituindo a decisão de tratamento mais ética (AKPATA et al., 1996; ARAÚJO et al., 1992; MEJÀRE, MALMGREN, 1986) e mais conservativa a ser tomada, evitando a necessidade de tratamento restaurador (ANGMAR MÄNSSON, TEN BOSCH, 1987).

Com métodos modernos, é possível detectar lesões em um longo estágio antes de se tornar cavitada, sendo muito importante realizar medidas preventivas apropriadas rapidamente. Além da detecção da cárie, fatores como o risco do paciente, número de lesões de cáries, experiência passada, dieta, presença ou ausência de fatores modificadores como a saliva, contagem de *S. mutans* e higiene oral, e ainda aspectos qualitativos como a cor e a localização anatômica da lesão (KIDD, 1998). No contexto destas mudanças, o diagnóstico de cárie depara-se criticamente com o impacto da decisão de tratamento, visto que diagnósticos incorretos resultam facilmente em tratamentos inadequados, particularmente com respeito à intervenção restauradora (TRANXEUS, SHI, ANGMAR-MÄNSSON, 2005).

Lesões cariosas ocorrem em uma variedade de locais anatômicos e apresentam características únicas de configuração e taxa de propagação. Essas diferenças mostram a impossibilidade de um único método diagnóstico ser adequado quanto a sensibilidade e especificidade para detectar todos os sítios de cáries. Múltiplos testes de diagnóstico aumentam a eficácia e precisão das novas

técnicas de diagnóstico de cárie, quanto à detecção e quantificação (HUYSMANS, LONGBOTTOM, PITTS, 1998).

O diagnóstico precoce pode indicar ou não uma terapia adequada, sendo o mais importante que a doença ou condição não se desenvolva antes de se tornar detectável pela tecnologia. Aprofundamento no conhecimento do processo carioso resulta em maiores exigências nas técnicas de diagnóstico. Nesse contexto conclui-se que a distinção entre a doença e não doença encontra-se muito mais relacionada ao estágio de avanços tecnológicos do que às características intrínsecas da doença.

Neste contexto, na apresentação de um novo método diagnóstico, pesquisas na odontologia precisam conduzir estudos que mostrem os rendimentos dos parâmetros básicos, tais como exatidão, precisão, sensibilidade, especificidade, valor preditivo positivo e negativo. Em seguida, se esses aspectos apresentarem-se aceitáveis para clínicos, sendo recompensadores para os clientes, parte-se para avaliação para determinar o custo-efetividade da nova tecnologia e demonstrar como a técnica poderá vir a alterar o processo saúde-doença, bem como a terapia a ser estabelecida para se obter a saúde do paciente (DOUGLASS, 1993).

Em uma breve revisão baseada em Douglas e McNeil (1983), definiremos alguns parâmetros, sendo os mais básicos a validação e confiabilidade, mais utilizadas na área de saúde para substituir os termos exatidão e precisão. A validação avalia se o teste verdadeiramente mede o que se propõe a medir, bem como mensura o nível de quanto o resultado é correto. A confiabilidade e precisão refletem a reproduzibilidade do método pelo mesmo e por outros avaliadores. Ambos os parâmetros são comparados a um padrão ouro pré-estabelecido para o método.

O diagnóstico no padrão ouro definitivamente independe da presença ou ausência da doença, levando-se em conta que são estabelecidos por resultados de biópsia e autopsia, usualmente pela histopatologia. Estes resultados serão padrões para avaliação dos outros parâmetros citados anteriormente.

A forma mais comum de expressar a acurácia de um método de detecção ou quantificação é através do cálculo da sensibilidade e especificidade, bem como analisá-los segundo a curva ROC (Receive Operator Characteristic Curve). A sensibilidade consiste na probabilidade de o teste fornecer resultados positivos quando a doença se faz presente (incluindo diagnósticos positivo verdadeiro - PV e falso negativo - FN). A sensibilidade é calculada a partir da fórmula $S = PV / PV+FN$; a especificidade consiste na probabilidade de o teste fornecer resultados negativos na ausência da doença (incluindo diagnósticos negativo verdadeiro - NV e falso positivo - FP), sendo portanto calculada pela fórmula $E = NV / NV+FP$.

A curva ROC é formada por vários níveis de alteração do teste, elaborando-se um gráfico da sensibilidade em função da proporção dos resultados falsos-positivos, sendo o resultado ideal, aquele que alcança a extremidade mais superior e esquerda do gráfico. Uma das vantagens deste método é que as curvas de diferentes testes diagnósticos podem ser comparadas; quanto melhor o teste mais perto estará sua curva do canto superior esquerdo do gráfico (SACKET, HAYNES, TUGWELL, 1991).

Embora o número de observações apresente impacto nos cálculos de sensibilidade e especificidade, o alto número de amostras analisadas promove um rendimento mais positivo quanto à performance do diagnóstico. (LUSSI et al., 2001). Os testes de sensibilidade e especificidade são independentes da prevalência da

doença, pois estes parâmetros são determinados apenas para pacientes que apresentam a doença (sensibilidade) ou para pacientes que não apresentam a doença (especificidade). Considera-se bem estabelecido desenvolver tal análise em estudos *in vitro*, sem cálculos amostrais prévios.

Em nossa pesquisa, em um dos artigos apresentados, realizamos teste de sensibilidade e especificidade quanto ao diagnóstico de cárie nas imagens realizadas por transiluminação com infravermelho próximo e pelo raio X digital, segundo observação de 3 profissionais cirurgiões-dentistas.

2.3 Propriedades Óticas Laser-tecido dentário

Nesta revisão, objetiva-se revisar a literatura quanto a informações relevantes da interação da onda eletromagnética com o tecido dentário e em seguida ressaltar alguns detalhes da operação dos métodos, bem como do potencial de vantagens e desvantagens de cada técnica. Dentre as que discutiremos, é interessante ter em mente, que estando em diferentes estágios de desenvolvimento, algumas delas possivelmente não serão aplicadas clinicamente para detectar cárie atualmente. Entretanto, o desenvolvimento contínuo de formas para se monitorar e compreender as interações da energia eletromagnética com o tecido dentário é atual e promissor para uso em outras técnicas (HALL, GIRKIN, 2004).

A onda de luz vem a tempo sendo utilizada como método de detecção de cárie. Previamente, os dentistas deparam-se na interpretação subjetiva de um sensor qualitativo e ajustável continuamente, como o olho. A detecção da cárie requer a observação das propriedades óticas da interação da luz aplicada no dente,

e neste contexto diferentes tecnologias vêm se expandindo na mensuração objetiva das seguintes propriedades: espalhamento, reflexão, absorção e fluorescência, ver figura 3 (HALL, GIRKIN, 2004).

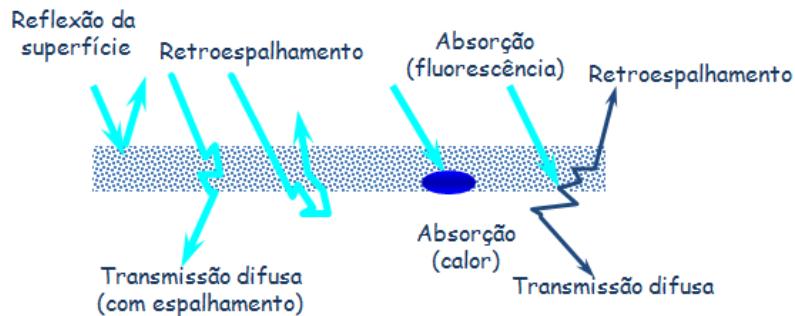


Figura 3: Esquema gráfico das propriedades óticas na interação laser-tecido.

O espalhamento é o resultado da mudança da direção da onda em simples ou múltiplas ocasiões, ao interagir com uma pequena partícula ou objeto de material não homogêneo, de dimensões da ordem do comprimento de onda da radiação utilizada. O ângulo e a quantidade do espalhamento dependem do comprimento de onda e do material, podendo levar a perda de energia, ou não. Dependendo do comprimento de onda incidindo no tecido dentário, esmalte ou dentina, a onda pode vir a espalhar e emergir do outro lado do tecido (fenômeno de transmissão), ser retroespelhada em direção à fonte, ou mesmo ser espalhada em quaisquer outras direções.

A reflexão é um fenômeno de superfície resultante da mudança de direção da onda após simples interação com o tecido, sendo esta alteração na direção oposta à onda incidente, não sendo alterada a energia ou onda de luz ou som. O ângulo de reflexão é igual ao ângulo de incidência.

A absorção requer que a onda seja absorvida pelas moléculas que compõem o tecido, transfira a energia absorvida para o tecido, convertendo-a em outras formas, como o calor. Um tecido pode, no entanto, absorver a energia da onda e convertê-la para outra onda de menor energia ou maior comprimento, sendo esta reemitida em uma onda maior de luz conhecida como fluorescência.

Contextualizando as propriedades óticas no diagnóstico de cárie, sabe-se que a perda mineral tem relação com o menor brilho refletido do mineral. Da mesma forma que as propriedades mecânicas e químicas, as ópticas são propícias a mudar com a desmineralização da microestrutura de tecido duro, sendo um dos primeiros sinais da cárie, as manchas brancas (JONES et al., 2006).

Particularmente, a luz é uma ferramenta utilitária para o estudo dos tecidos dentários. A estrutura regular permite boa propagação da luz através do esmalte cristalino e dos túbulos da dentina. Rupturas na estrutura organizada dos dentes aumentam o espalhamento da luz ao atravessar o dente (ROUSSEAU et al., 2007). Bem como, o aumento de fluidos dentro dos poros formados pelo processo de desmineralização, associado a manchas exógenas, produtos finais bacterianos entre outras contaminações presente no processo carioso, levam a alterações na interação normal da luz com a estrutura dentária.

A interferência do comprimento da onda é indiscutível na interação com o tecido, particularmente para absorção e espalhamento. A probabilidade de espalhamento diminui com o aumento do comprimento de onda, concluindo-se que longos comprimentos de onda espalham menos que os curtos, e consequentemente penetram mais profundamente no tecido.

2.4 Técnicas Ópticas de Diagnóstico

O caminho para se chegar ao diagnóstico é o exame clínico e os exames complementares (TOMASI, 2002). O primeiro é considerado soberano devido à possibilidade de se obter um grande número de informações importantes sobre o paciente. O segundo auxilia o profissional, confirmando hipóteses de diagnóstico geradas durante o exame clínico, e o orienta na tomada de decisão para o melhor tratamento. No que diz respeito aos exames complementares, novas tecnologias estão sendo utilizadas e aperfeiçoadas para o uso em diagnóstico por imagem. É necessário, então, estar atento ao seu emprego e aos benefícios que essas tecnologias proporcionam.

Embora seja difícil acompanhar a evolução dos métodos de diagnóstico, o profissional deve conhecer as opções de técnicas e, principalmente, a aplicabilidade e eficiência desses métodos no diagnóstico precoce e plano de tratamento mais adequado (AMORE et al, 2000).

Considerando que a meta no diagnóstico de cárie consiste na detecção precoce, bem como definição quanto a atividade da lesão, nenhum dos métodos disponíveis pode ser apontado como solução, com exceção da combinação de métodos visuais com a experiência clínica do paciente, o histórico da dentição, e outros fatores como o fluxo salivar e a capacidade tampão. A atividade da lesão pode ser bem determinada por várias avaliações (AL-KHATEEB et al., 1998).

A maioria das técnicas discutidas atualmente lança mão da interpretação dessas diferentes propriedades físicas geradas a partir do processo de interação da luz com o tecido. Uma determinação detalhada dos parâmetros óticos do tecido geralmente requer uma completa caracterização das propriedades de espalhamento e absorção do tecido. Para desenvolver efetivamente tecnologias e otimizar a

eficiência das técnicas, é imprescindível o entendimento de como a radiação eletromagnética na região do espectro óptico propaga pelo tecido dentário e como as propriedades óticas do dente mudam em consequência de um processo de desmineralização natural ou carioso do dente (DARLING, HUYNH, FRIED, 2006).

Na década de 90, algumas pesquisas têm demonstrado boa perspectiva relacionada à utilização da fluorescência induzida por laser como auxiliar no diagnóstico da cárie (EMAMI et al., 1996; DE JOSSELIN DE JONG et al., 1995), comprovando que com a ajuda do laser torna-se capaz de detectar desmineralizações iniciais, principalmente quando é adicionado corante fluorescente na superfície dental que se quer avaliar (FERREIRA-ZANDONÁ et al., 1998). A utilização do laser diodo (DIAGNOdent) mostrou-se inicialmente ideal para detectar desmineralizações iniciais, com o inconveniente de apresentar alto número de diagnósticos falso-positivos (GARCIA, ARAÚJO, TOVO, 2000; PARDI et al., 2000; RIBEIRO, 2001).

O uso de comprimento de onda longo auxilia em técnicas de diagnóstico devido à maior penetração através do tecido, mas por outro lado a resolução da imagem é diretamente proporcional ao comprimento de onda. Significando que quanto maior o comprimento de onda usado, mínima resolução possível é obtida. Em um sistema óptico, a combinação de lentes com o comprimento de onda para receber a energia determina o limite de resolução. Como um guia grosso, excluindo técnicas ópticas sofisticadas, fazer imagem com uma resolução bem menor que o comprimento de onda da luz é difícil. Sistemas de imagem de microscopia, usado em estudos histológicos, podem apresentar resoluções em torno de 0,3um utilizando lentes objetivas com óleo e trabalhando em pequenas

distâncias, o que seria uma dificuldade para os instrumentos clínicos de diagnóstico dental. Um exemplo facilitador, consiste no sistema de ultra som, que com ondas significativamente longas, apresentam limite resolução muito baixo (HALL, GIRKIN, 2004).

Num contexto geral, a resolução dos sistemas óticos não é normalmente o fator limitante do tamanho do detalhe que pode ser visto. Não há vantagem em conseguir a mais alta resolução ótica possível se não há contraste na imagem. O contraste é definido como a diferença entre pontos de luz claros e escuros, e se esta diferença não pode ser observada, provável que o observador não consiga resolver a estrutura. Geralmente, a perda de contraste nos sistemas óticos de imagem é causada por eventos de espalhamento, desviando a luz para pontos errados no detector.

Quanto aos cuidados de biocompatibilidade, Otis et al. (2000) observaram em seu estudo que, de acordo com a ANSI (American National Standards Institute), é necessário uma exposição da mesma fonte, por exemplo com comprimento de onda de 1,3 μm e uma potência de 96 mW, por 8 horas seguidas para haver dano à pele humana.

Alguns métodos não apresentam aplicação clínica, visto que podem destruir o dente na busca por cáries, outros serão limitados a dentes, ou superfícies restritas de um dente. Mas pode-se afirmar que as utilidades e limitações da maioria dos métodos são definidas na compreensão de como as fontes de energia luminosa interagem com o tecido dentário. Dentre as técnicas citadas inicialmente, tivemos a oportunidade de explorar maiores detalhes diretamente das técnicas de transiluminação com infravermelho próximo comparando com o raio-x, bem como explorar as características quantitativas da Tomografia por Coerência Óptica.

2.4.1 Raio X

Ao abordar o diagnóstico de cárie, a Radiologia Odontológica demonstra-se essencialmente bioética, com a busca incessante de conhecimentos que culminem em uma menor dose de radiação aplicada ao paciente aliada à qualidade dos dados obtidos. Exemplo deste propósito é o desenvolvimento de filmes de sensibilidade crescente, a despeito da inexorável e tão discutida perda da nitidez da imagem (HINTZE, CHRISTOFFERSEN, WENZEL, 1996). Nas duas últimas décadas, ao incorporar recursos da computação digital, a Radiologia Odontológica demonstrou uma notável diferenciação tecnológica e potencializou sua condição de um importante recurso diagnóstico auxiliar (VERSTEEG, SANDERINK, STELT, 1997; WENZEL, 1998).

A imagem radiográfica interproximal permite condição informativa diferenciada e indispensável para a avaliação do paciente, quando se perscrutam lesões da doença cárie localizadas em superfícies dentárias contíguas, inacessível ao diagnóstico clínico visual. As radiografias interproximais têm sido aceitas como o método mais comum e útil para o diagnóstico de cáries proximais (CORTÊS, 1998), sendo adequado para detectar cárie oculta em dentina, mas falho para lesões em esmalte (ZANARDO, REGO, 2003). Somado a uma série de contra-indicações, como a exposição do cirurgião dentista, de sua equipe e dos pacientes à radiação ionizante, muitas outras técnicas vem sendo desenvolvidas utilizando-se a luz/laser como fonte.

Entretanto, mesmo as imagens radiográficas não conseguem traduzir se a lesão está paralisada ou em atividade, impossibilitando a observação da involução da doença em casos de técnicas não invasivas (BARATIERI et al., 2002), além de

que, apresentam problemas técnicos (como superposição de proximais e deficiência de contraste), subestimação da lesão e inabilidade em diferenciar lesões proximais pré-cavitadas daquelas cavitadas (ZANARDO, REGO, 2003).

Atualmente, as imagens radiográficas podem ser obtidas por métodos digitais diretos, os quais dispensam a utilização de filmes (MOORE, DOVE, McDAVID, 1998). São incipientes os estudos nacionais que tenham por objetivo avaliar o diagnóstico de lesões de cárie por métodos digitais (KERBAUY, MORAES, 1996).

2.4.2 Transiluminação com Infra Vermelho Próximo

As restrições e limitações da radiação ionizante discutidas, consistem no estímulo da investigação dos sistemas ópticos para obtenção de imagens que possam detectar lesão de cárie inicial, promovendo conjuntamente compatibilidade biológica, visto que esses métodos, ao contrário dos exames radiográficos, não apresentam efeitos colaterais (MIALHE et al., 2000), no que diz respeito aos comprimentos de onda adequados que possibilitam uma avaliação freqüente do paciente. (JONES et al., 2003).

Nos 30 anos passados, o desenvolvimento de altas e intensas fontes de iluminação por fibras óticas resgatou o método para a detecção da cárie (JONES et al., 2003). Além da biocompatibilidade, alguns métodos de diagnóstico com luz priorizam: resultados confiantes; detecção de lesões num estágio inicial; diferenciação de lesões reversíveis das irreversíveis e documentação. Várias tentativas foram feitas no sentido de melhorar os métodos tradicionais ou de criar

novos sistemas de diagnóstico, como transiluminação por fibra ótica, (FOTI), que tem se mostrado promissor na detecção da cárie (CORTÊS, 1998).

Buscando-se sempre melhorar o diagnóstico, foi desenvolvido o sistema de análise digital, a transiluminação por fibra óptica com imagem digital (DIFOTI) com luz visível apresentando boa especificidade, mas sua sensibilidade é similar à da inspeção visual (PARDI, 2000; VERDONSCHOT, 1992), produzido pela Electro-Optical Sciences, recentemente aprovada pela FDA. Baseado no princípio da transiluminação dental de acordo com a mineralização de diferentes áreas do esmalte e dentina, o aparelho detecta cárries incipientes que aparecem escuras na imagem formada através das duas pontas de mão bem posicionadas que capturam a imagem para programa de computador.

Entretanto, o grande espalhamento da luz sobre o esmalte dental quando usando comprimentos de onda visíveis, 400-700 nm reduz fortemente a imagem formada através do dente. Estudos prévios têm mostrado que a magnitude do espalhamento decresce exponencialmente para comprimento de ondas longo, como observado na figura 4 (FRIED et al., 1995; SPITZER, TEN BOSCH, 1975). A atenuação dos coeficientes de extinção (absorção+espalhamento) do esmalte dental mede em torno de $3,1\text{cm}^{-1}$ e $3,8\text{cm}^{-1}$ quando em comprimentos de onda de 1310nm e 1550nm, respectivamente. A magnitude do espalhamento nesses comprimentos de onda é mais que 30 vezes menor quando comparado à escala visível (JONES, FRIED, 2002).

Baseado nesses dados, Jones et al. (2003) demonstraram, ao analisar cárries artificiais em diferentes espessuras de esmalte dentário, que o resultado da transiluminação no infravermelho próximo apresentava contraste superior a outras

técnicas como o raio x e a transiluminação no visível, como pode ser observado na Figura 5.

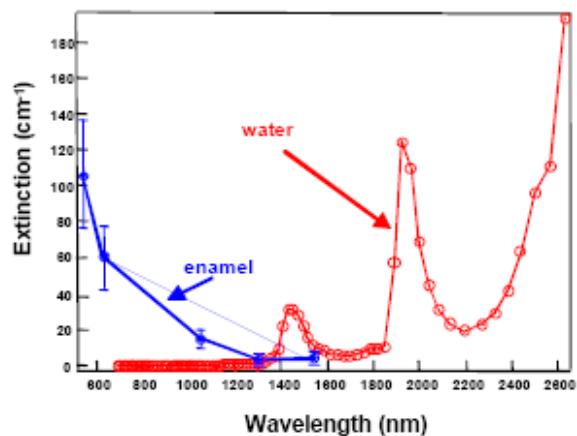


Figura 4: Gráfico do coeficiente de extinção da luz no esmalte e água em função do comprimento de onda.(Gráfico de Jones et al., 2003).

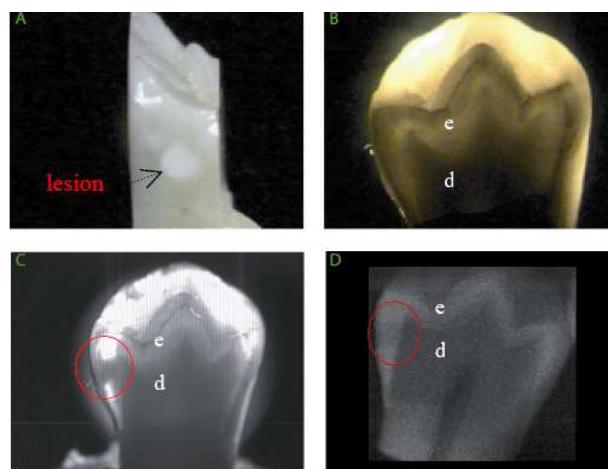


Figura 5: Exemplo de uma amostra de 3mm de espessura com uma lesão de cárie simulada. B) Imagem da transiluminação com luz visível, C) Lesão claramente visível com transiluminação no infra vermelho próximo, D) Raio x com filme D-speed mostrando baixo contraste entre o esmalte sadio e a lesão. Em todas as projeções foi possível distinguir a esmalte sadio e a dentina. (Imagens de Jones et al., 2003).

Baseado nas pesquisas de Jones et al., (2003), foi demonstrado que a luz em torno de 1310nm funciona bem para detecção e imagem das lesões cariosas proximais, pois o esmalte apresenta-se extremamente transparente enquanto sadio.

O sistema montado, segundo esquema da Figura 6, consistiu em quatro diferentes fontes de diodos superluminescente, com dois polarizadores perpendicularmente cruzados entre si, para bloquear a luz ambiente ao redor da amostra, a ser detectada pela CCD com detector de espectro entre 400nm e 2000nm. Esforços foram realizados para o completo alinhamento do sistema polarizadores/lentes para que a luz atravessasse perpendicular a fatia dentária, e um difusor foi posicionado atrás da amostra para despolarizar a luz uniformemente através do dente.

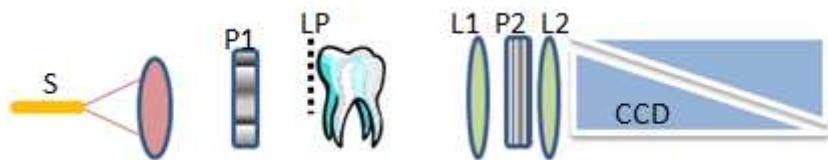


Figura 6: Esquema Transiluminação com Infra-Vermelho Próximo consiste em uma fonte de luz com largura de banda (S), dois polarizadores (P1) e (P2) cruzados perpendicularmente, combinação de lentes (L1) e (L2), e a CCD. Durante o teste a máscara de linhas de pares (LP) foi inserida.

Nestas circunstâncias optamos por explorar mais esta metodologia utilizada, alterando algumas variáveis, para avaliação da resolução ótica e informações quanto à sensibilidade e especificidade da técnica.

2.4.3 Tomografia por Coerência Óptica

Huang et al. (1991) trouxeram uma importante contribuição para a Biomedicina ao propor um novo método de diagnóstico: Tomografia por Coerência Óptica (TCO). Eles foram os primeiros autores que descreveram esta técnica como um meio de diagnóstico para tecidos biológicos, pois até então ela era somente empregada na indústria de telecomunicações para identificar e caracterizar reflexões nos componentes ópticos. O sistema desenvolvido por Huang e colaboradores baseou-se no interferômetro de Michelson e utilizou como fonte de luz o diodo superluminescente de baixa coerência emitindo um comprimento de onda médio de 830 nm. Por se tratar de um método digital, medidas quantitativas precisas podem ser obtidas podendo-se calcular a espessura dos tecidos através da multiplicação do atraso sofrido pela luz, pela sua velocidade neste tecido, a qual depende do índice de refração do tecido e da velocidade luminosa no vácuo.

Dois domínios podem ser explorados pra implementar o sistema de Tomografia por Coerência Óptica: o domínio de tempo (TD-TCO) e o domínio espectral (SD-TCO). No domínio do tempo como mostrado na Figura 7, o braço do atraso da linha de luz basicamente consiste tanto no braço em movimento como em um atraso por domínio de Fourier (CENSE, NASSIT, CHEN, 2004). No domínio espectral, Figura 8, não há partes móveis no braço de interferometria e a recombinação dos feixes que partem do interferômetro são enviados ao espectrômetro e análise de Fourier. O sistema SD-TCO tem algumas vantagens sobre o TD-TCO, incluindo sensibilidade (WOJTKOWSKI, KOWALEZYK, 2002) e rápida aquisição dos dados.

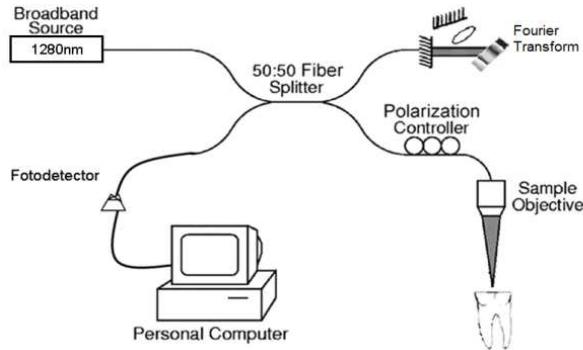


Figura 7: Diagrama esquemático do sistema de tomografia por coerência óptica TD-TCO operando em 1280nm com Domínio de Fourier com linha de atraso.

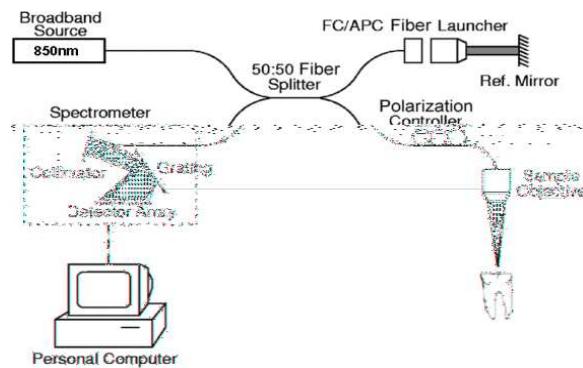


Figura 8: Diagrama esquemático do sistema de tomografia por coerência óptica. SD-TCO operando em 850nm com Domínio espectral.

Em Odontologia, as primeiras aplicações começaram em 1998 (COLSTON et al., 1998) e, atualmente, tem sido utilizada para diversos fins como avaliação da interface de restaurações em esmalte (MELO et al., 2005), para realizar correto diagnóstico de cárie (FREITAS et al., 2006), para análise da performance de materiais dentários (KYOTOKU et al., 2007; BRAZ et al., 2008), detecção precoce de câncer oral (JUNG et al., 2005), detecção de cáries recorrentes e adaptação marginal de restaurações (OTIS et al., 2000), caracterização de estruturas periodontais (COLSTON et al., 1998; OTIS et al. 2000). Em 2006, Kauffman et al., estudaram a primeira imagem de TCO da polpa dental utilizando dentes de rato.

Análises de lesões de cárie foram realizadas e mudanças nos sinais ópticos estão relacionadas ao espalhamento e possível grau de desmineralização, o que

como foi discutido, implica em diagnóstico não invasivo de cárie precoce e secundária (HALL, GIRKIN, 2004).

A resolução das imagens obtidas a partir da técnica de TCO está relacionada à fonte de luz empregada no sistema. O comprimento de coerência, por exemplo, delimita a resolução axial do sistema, que, por sua vez é inversamente proporcional à largura de banda da fonte. Outro aspecto importante relacionado à fonte de luz empregada é o comprimento de onda, pois dele depende a capacidade de penetração do feixe na amostra estudada (MELO, 2005).

Comparando a capacidade de penetração de dois comprimentos de onda e utilizando como fonte de luz o diodo superluminescente, Pierce et al. (2004) também comprovaram uma melhor penetração do laser em 1300nm comparado ao de 800nm na pele. Já Welzel et al. (1997), utilizando o comprimento de onda de 830nm, visualizaram profundidade de 0,5 a 1,5mm na pele humana.

Muitos autores reforçam este resultado afirmando que a região espectral atrativa para imagens da TCO em tecidos biológicos não transparentes é próximo de 1.3 μm , onde o espalhamento da luz é relativamente baixo comparado ao espalhamento de luz na região visível, e a absorção tecidual é menor. Neste comprimento de onda próximo a 1.3 μm , muitas investigações têm demonstrado imagens de 1-3mm de profundidade, dependendo do tipo de tecido examinado (BREZINSKI; FUJIMOTO, 1999; FUJIMOTO et al., 2000).

Feldchtein et al. (1998) realizaram um estudo onde investigaram a formação de imagem por TCO *in vitro* e *in vivo* das estruturas de tecidos duros e moles da cavidade oral. O sistema de TCO utilizado tinha como fonte de luz diodo superluminescente operando em 830 nm e 1280 nm com uma resolução axial no primeiro de 13 μm e no segundo de 17 μm . Nos tecidos duros, os autores

investigaram a habilidade do TCO em formar imagens de lesões cariosas e não cariosas em esmalte e dentina nas áreas cervical, proximais e sulcos oclusais de vários dentes, além de avaliar a qualidade das restaurações ao verificar fendas entre o material restaurador e o dente, bolhas de ar incorporadas durante a inserção do material e outros defeitos que determinassem a sua substituição.

Otis et al. (2000) realizaram imagens *in vivo* de estruturas dentais sadias em adulto com um sistema de TCO desenvolvido e testado por eles que apresentavam 140 microwatt de potência, 1310nm, diodo superluminescente como fonte de luz, 70 femtowatts de luz refletida e profundidade de imagem de aproximadamente 3mm. Os autores consideraram que o TCO tem a capacidade de revelar detalhes da estrutura dental normal e quantificar os principais problemas dentários como cáries, podendo ser utilizada na proservação da doença.

Jones et al. (2006), utilizando a tomografia por coerência óptica com polarização sensitiva, investigaram a relação entre a magnitude do retroespalhamento da luz e da despolarização reconhecida pelo mesmo na avaliação de mudanças no volume mineral em modelos artificiais de cárie. O sistema de PS-TCO tinha como fonte de luz dois diodos superluminescentes um com 26mW não polarizado e então com output final de 13mW, e o outro linearmente polarizado com 20mW pouco atenuado. Ambas as fonte com largura de banda de 50nm, produzindo uma resolução axial no sistema de 20 μ m. Os resultados foram comparados a digital microradiografia de alta resolução, com alta correlação entre os métodos. Confirmado que a TCO com polarização sensitiva pode captar imagem e quantificar através da medida do aumento do espalhamento e despolarização do laser infravermelho próximo.

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ARTIGOS REFERENTES À DISSERTAÇÃO

Como resultado desta dissertação, os seguintes trabalhos serão submetidos a publicação:

ARTIGO 1

Lena Karlsson, Ana M. A. Maia, Bernardo B. C. Kyotoku, Walter Margulis, Anderson S. L. Gomes. **Evaluation of Near-Infrared Transillumination System for Early Caries Detection.** A ser submetido ao Journal of Biomedical Optics.

ARTIGO 2

Ana M. A. Maia, Lena Karlsson, Walter Margulis, Anderson S. L. Gomes. **Evaluation of sensibility and specificity of near infrared transillumination and radiographic images of noncavitated caries lesions.** A ser submetido a Photomedicine and Laser Surgery.

ARTIGO 3

Ana M. A. Maia, Déborah D. D. Fonsêca, Bernardo B. C. Kyotoku, Anderson S. L. Gomes. **In Vitro characterization of enamel in deciduous teeth by Optical Coherence Tomography: comparison between 850nm and 1280nm.** A ser submetido para International Journal of Paediatric Dentistry.

Além destes artigos, paralelamente foram publicados e/ou submetidos, bem como apresentados em congressos os seguintes trabalhos:

MAIA, A.M.A., BARKOKEBAS, A., PIRES, A.P., BARROS, L.F., CARVALHO, A.A.T., LEÃO, J.C. Current use and future perspectives of diagnostic and therapeutic lasers in oral medicine. *Minerva Stomatol*, 57: 2008. Aceito para publicação.

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Evaluation of Near-Infrared Transillumination System for Early Caries Detection

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Abstract

Near infra-red light transillumination (NIR-TI) has been used as a method of caries detection and as current and future technology. The objective of this research was evaluated the spatial resolution of the system, making possible to observe how early a caries lesion can be detected by NIR-TI; elucidated possibilities and limitation of the technique, and calculated the real position of the caries lesion. The interference of light propagation on different enamel thickness and the propagation of a resolution mask through the teeth was analyzed. Our results showed the visualization of caries in a quantitative way, and proved that a system's ultimate resolution is not normally the factor limiting the size of features that can be seen. As expected, it was also observed that the contrast plays a very important role in the image.

Introduction

In the search for more accurate diagnostic approaches, investigators have used alternative non-invasive and instrument-based techniques for detecting and quantifying dental demineralization lesions [1,2,3] that would be amenable to remineralising therapies [4]. Most of them are based on the interpretation of physical signals, particularly photonics based effects [5], like quantitative light fluorescence (QLF) [6], laser-induced fluorescence [7], optical coherence tomography (OCT) [8], Raman spectroscopy [9,10], digital imaging fibre-optic transillumination (DIFOTI) [11] and Near infrared transillumination [12,13].

To effectively develop near infrared (NIR) technologies and optimize their effectiveness, it is necessary to understand how NIR light propagates through the tooth and how the optical properties of the tooth change as a result of natural demineralization or tooth decay [14].

The DIFOTI method [11] uses high intensity white light that is shone through the tooth. The technique essentially picks up surface scattering and renders the possibility of visually discriminate between enamel and dentine caries. The image can be captured, saved and stored as a digitalized version, with a subjective interpretation of the appearance of the lesion. Despite encouraging results from its forerunner FOTI, and that the method is commercially available only limited research has been performed so far. One possible explanation as the factor limiting caries detection via optical imaging methods in the visible light spectrum scattering that is sufficiently strong to obscure the light transmission [14].

Methods employing shorter wavelengths within the visible range of the electromagnetic spectra (400-700nm) such as QLF ($\lambda < 520\text{nm}$), the LF method ($\lambda = 655\text{nm}$) and DIFOTI, cause scattering events when interacting with the dental hard tissue, while the novel and promising method, the near-infrared (NIR) transillumination system with longer wavelengths (1300nm and 1400 nm) scatters less than shorter wavelengths and therefore can penetrate the tissue more deeply. Previous research by Jones and Fried (2002) [15] measured the optical attenuation of NIR light through polished thin sections (0.1 to 2.5mm) of dental enamel at 1310nm and 1550nm. The collimated transmission was measured in cuvettes of index matching fluid with $n = 1.63$, and show that the attenuation coefficients are $3.1 \pm 0.17\text{cm}^{-1}$ and $3.8 \pm 0.17\text{cm}^{-1}$ for 1310nm and 1550nm, respectively. The magnitude of scattering, a process in which the direction of photons is changed without loss of energy [16], at those wavelengths is more than a factor of 30 times lower than in the visible range. This translates to a mean free path of 3.2mm for 1310nm photons [15].

Early caries imaging with NIR light at 1310 nm has demonstrated considerable potential since enamel tissue is highly transparent in this region of the electromagnetic spectrum [13, 14, 15, 17]. Increased backscattering from the demineralized region of early caries lesions is the basis for the visual appearance of white spot lesions [18, 19]. An increase in porosity of the lesion leads to increased scattering at the lesion surface and higher scattering in the body of the lesion, producing an increase in the magnitude of the diffuse reflectance [20].

As Hall and Girkin [16] pointed out, there is a inverse relationship between the wavelength used and the resolution of the image. Longer wavelengths may penetrate more deeply within the tooth but is at same time inversely related to the spatial

resolution; longer wavelengths give less information of smallest resolved detail that can be seen.

Assessing the resolution of acquired images objectively is important and is useful in a (clinical environment) to quantify the best possible spatial resolution of the TI technique in order to estimate how early a caries lesion can be detected. Spatial resolution refers to the ability to sharply and clearly define the extent or shape of features within an image, or the ability to distinguish between two closely spaced objects on an image [21] e.g. caries tissue in comparison to surrounding healthy tissue.

In this paper, the spatial resolution and contrast of the near-IR transillumination on early caries detection was studied by simulations with serials line pairs per mm gauges on different tooth thicknesses. Subsequently, a sample with artificial caries demonstrates how its contrast changes when the tooth image is captured from both sides of the sample.

Materials and Method

We collected three human permanent first molars extracted at the Human Teeth Bank of the Departament of Prohest and Dental-Facial Surgery of the Universidade Federal de Pernambuco, after the Ethics Committee approved the study (268/2007). Immediately all teeth were stored in a physiological saline solution 0,9% to preserve tissue hydration.

Based on the methodology described by Jones et al. (2003) [15], a similar system was built at the Optoelectronics and Photonics Laboratory at the Universidade Federal de Pernambuco, Brazil. The basic experimental scheme is

illustrated in Figure 1. We demonstrate the application of TI system with two wavelengths: 1280nm SLD-571 (model SLD, SUPERLUM, Moscow, Russia) with an output power of 5mW and a bandwidth of 64,6nm; 1400nm Raman (Raman Fiber Laser, Key Optical System, China) with an output power of 50mW and a bandwidth of 0,6nm;

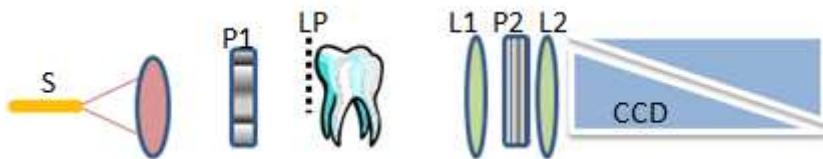


Figure 1: NIR Transillumination set up consists of a broadband light source (S), crossed linear polarizers (P1) and (P2), lenses (L1) and (L2), and the Micron Viewer IR camera (CCD). In the test a line pair gauge (LP) will be inserted.

Two crossed polarizers were placed perpendicular to each other to experimentally block out the ambient light from saturating the detector; a CCD camera with spectral response characteristic at 400 nm to over 2 μm (MicronViewer 7290A, Electrophysics, Fairfield, NJ, USA) was used. Due to the natural tooth contours, efforts were made to assure that the light travelled perpendicular to the analyzed sections. A diffuser was used behind the sample to depolarize the illuminated light uniformly around the tooth. The 32-bit digital images were captured using Spiricon Laser Beam Diagnostics (LBA-PC, Version 2.5, Utah, USA) and analyzed with a downloadable image processing program, ImageJ (NIH, Maryland, USA).

To evaluate the spatial resolution of TI Systems, we focus on transmittance of intact enamel *in vitro* and the ultimate resolution possible. Rather than working with caries tissue of undetermined contrast, a line pair gauge, printed in a photolithograph

by a Graphic Imagesetter, set by the US Air Force was used to measure image resolution. The gauge had totally dark and transparent lines that are close together and periodic across position in space, measured in line pairs per millimeter (lp/mm). Working with a line pair replacing the image of an absorber and the adjacent lucent space, six different masks were used, ranging from 5lp/mm through 0.83lp/mm that represents carious dimensions with 100 μ m and 600 μ m, respectively, projected through the tooth to evaluate the resolution of the system.

All samples had both mesial and distal faces cut off, with a Low Speed diamond Whellsaw, model 650, SBT, inc. The experiment started with three plan-parallel sections with 6 and 7mm of thickness, but two of them with sound interproximal faces. The both thick sections were imaged perpendicular to the occlusal surface and all sizes of the charts were set-up according to figure 1. Each image was captured twice due to the tooth morphology; once with the chart positioned horizontally and once with the chart positioned transversally. The samples were then sectioned by a Micro Electrical Motor, (Beltec, LB 100 Model, São Paulo) consecutively into 6, 5, 4, 3, 2 and 1 mm and the image capturing procedure was repeated for each thickness.

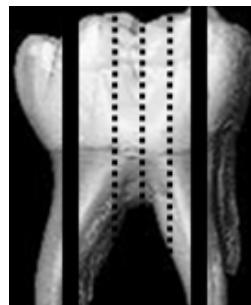


Figure 2: Model of mesial and distal faces cut off and consecutively longitudinal sections.

Another point analyzed was the importance of the location of the caries lesion and whether the contrast differs when signals have to transverse a thick part vs. a thinner part of the tooth when illuminated. A simulated enamel lesion was prepared in a sound human tooth closer to the lingual face by drilling, a 1-mm diameter and 1-mm deep cavity, with a diamond bur round 1012 (KG Sorensen, São Paulo), and filling it with calcium hydroxide cavity lining material (Dycal®). It was subsequently sealed with a thin layer of resin composite Filtek Z 350, color A2 (3M ESPE, São Paulo).

The incident light for TI was projected onto the section, acquired from both sides of the sample with signal(s) transmitted from the sample with the lesion located near the CCD camera and second with the lesion located far from the camera. The sample was then reduced consecutively on one side by the same Micro Electrical Motor, (Beltec, LB 100 Model, São Paulo) into 5mm, 4mm, 3mm and 1,8 mm sections and the image capturing procedure was repeated for each thickness.

Therefore, all images were analyzed regarding the Contrast (C) that was calculated with a downloadable image processing program (ImageJ, NIH Bethesda, Maryland, USA) by plotting the profile of intensity values of pixels along a line, set on a designated area of all images. The contrast was defined as $C = I_{\max} - I_{\min} / I_{\max}$, where I_{\max} is maximum intensity of the peaks and I_{\min} is the lowest value. After an interval of 1 week, the same observer repeated the procedure for assessing the intra-examiner reproducibility, evaluated by Lin's Intra-class Correlation Coefficient (ICC) [22].

Therefore, by performing these two measurements on both surfaces of the tooth thickness, all images were compared with respect to the contrast, making possible to estimate how close to one of the proximal face the caries was. If the caries is equidistant from proximal faces, the contrast should be equal.

Results

The line pair gauge, used as a “perfect” periodic caries lesion pattern was placed behind intact teeth of various thicknesses and the imaging light passing through both mask and enamel tissue, and detected by the CCD, as shows in figure 3. As discussed before, the resolution power was measured as the highest number of lp/mm that could be distinguished on the resultant image for a given contrast. The line pairs contrast projected through the tooth was calculated for all different enamel thickness, comparing 4 points of maximum value and 4 points of minimum value in a straight line. The mean of I_{\max} and I_{\min} , was calculated as mean peak intensity, and mean lowest intensity, respectively. And results of $(I_{\max} - I_{\min}) / I_{\max}$ were plotted.

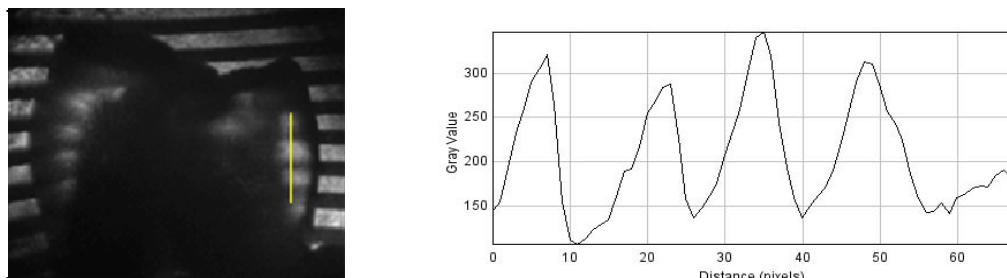


Figure 3: Shows the projection horizontally of one line pair gauge with 2,5 lp/mm through a thickness of 4mm; In the interproximal faces, contrast peaks can be evaluated by maximum and minimum averages.

All mean values calculated by contrast of the line pairs projected through the tooth were graphically plotted (figure 4), comparing thickness and wavelength. In general, our samples show higher enamel translucence at 1400nm than at 1292nm.

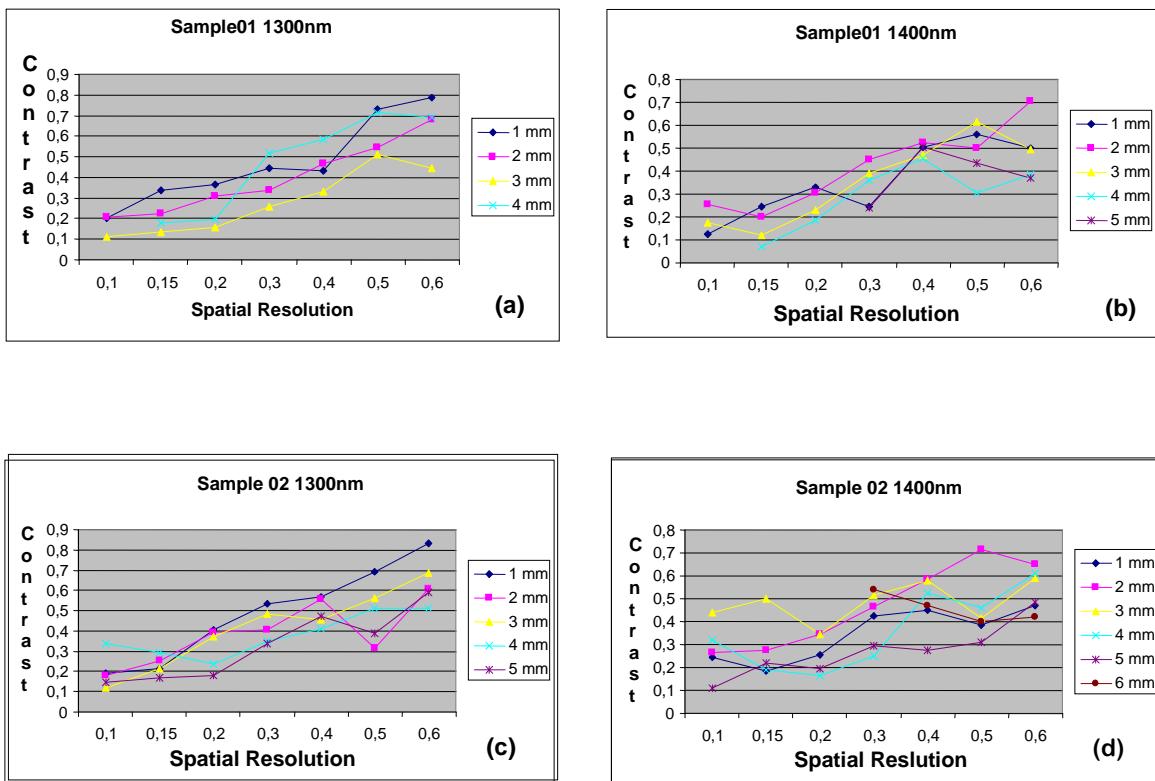


Figure 4: Graphic distribution of mean values of contrast by different masks and six thicknesses, at both wavelengths, showed a similar comportament.

In sequence, another series of experiments were performed to evaluate the propagation of the light through different thicknesses of enamel, which had a carious lesions. Due to the anatomic form of a tooth with 5 “faces”, we named the vestibular face A1, lingual face A2, proximal faces B1 and B2, and occlusal surface C1. The transillumination set-up was assumed to have the light source from face A2, and the detector looking at the tooth from face A1, and vice-versa, as show in Figure 5.

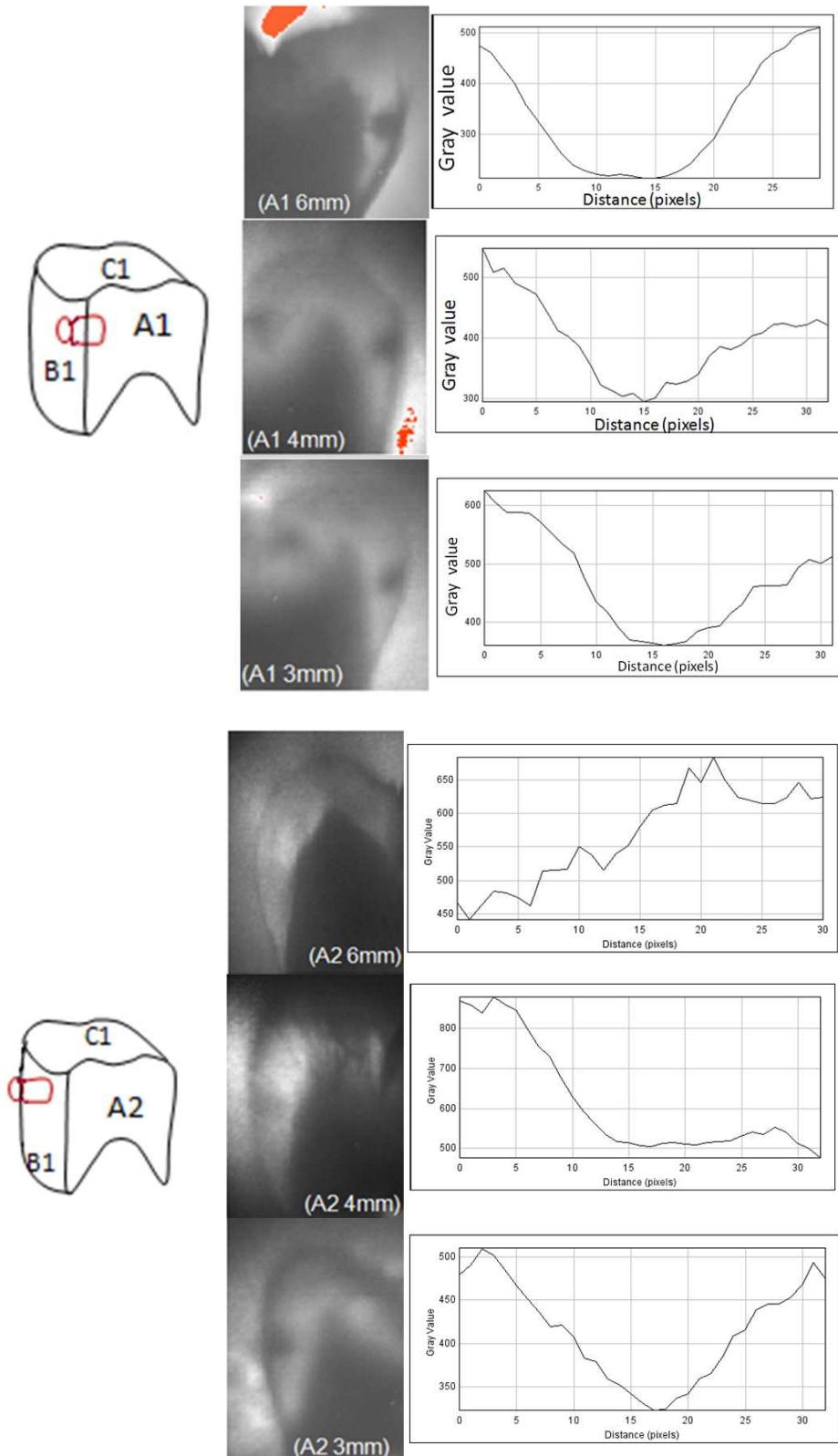


Figure 5: Evaluation of caries lesion contrast comparing images from both sides of the same sample. In the first sequence, caries face is near the CCD; In the second sequence, caries face is far from the CCD. The graphics with each image shows the contrast variation.

It was possible to observe a good contrast when the caries was near the exit surface of the tooth (closer to the CCD detector). But, on the other hand, while the tooth was turned around it, and light source illuminated the first face A1 and the detector camera collected the image from the face A2, and the contrast was poor, because the signal with the high contrast would have to traverse a thick part of the tooth before exiting towards the camera.

Looking at the graphs, it is possible to observe that the contrast drops with the thickness of enamel traversed. By performing measurements on both sides, it is possible to estimate how close to each surface the caries is. If the caries are equidistant from both faces, the contrast should be equal.

Discussion

The transillumination (TI) method is a non-destructive technique and more sensitive to early demineralization than x-rays [14] and without the use of harmful radiation. The advantage of the TI system could therefore be a decrease of the amount of dental x-rays taken and the ability to use the TI more frequently for monitoring caries regression or progression.

The resolving power is measured as the highest number of line pairs per millimeter that can be distinguished on the resultant image. Traditional film displays higher resolution than digital detector, 16-20 lp/mm and 8-10 lp/mm respectively [23]. However, the quality of an image depends on much more than the spatial resolution. The efficiency of an imaging system is important and encompasses several parameters such as brightness, contrast, blur etc. Images produced by the same signal may have completely different visual appearance, information, and

characteristics on different display devices. One important reason for this problem is that electronic displays – irrespective of the technology used - show a very variable behaviour in converting electric input values to luminance.

Clearly, this is unwanted in medical applications where the image and consequently, the diagnosis needs to be invariable and consistent, independent of which hardware was chosen to render the electronic image. The resolution was estimated using the ImageJ software; one observer analyzed all images two times during one week, in the same conditions.

The optical constant parameters as the absorption and scattering coefficients for sound enamel have been reported at many different wavelengths [14,15], which characterizes light transport in dental hard tissue, making it possible to determine the optical alterations when caries is present.

For enamel the results indicate that the hydroxyapatite crystals contribute significantly to scattering and that the influence of the prism structure on the light propagation is small [24]. Based on Hunter Schreger bands phenomenon [25], it's known that arrangement of enamel prisms in different types of enamel/enamel prisms do not follow a straight course, but are undulating in the horizontal plane as well as displacement in the vertical plane. This undulation results in the appearance of alternate bright and dark bands, which extend to the inner two-thirds of the enamel.

One interesting point calculated in our experiment was the range of contrast when the artificial caries lesion was near or far from the CCD, and the central importance to this application is the possibility of false negative results during clinical evaluation. To solve this problem translation and rotation of the CCD is necessary and the real position of the caries by overlapping contrast from both images must be estimated.

Conclusion

The TI method enables imaging and visual detection of dental caries on approximal surfaces - surfaces where the majority of caries lesions appear - and presents a clinical recognisable image with visual description which is preferable for average clinicians. The TI system can be built as a dental hand piece for intra-oral use, but unfortunately the technical advanced equipment of NIR CCD required for the system to be used on dental clinics.

Acknowledgements

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Evaluation of sensibility and specificity of NIR TI and X Ray images of noncavitated caries lesions

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Abstract

An increase in research activity surrounding diagnostic methods of early carious lesions detection is a prerequisite to establish clinical parameters of diagnosis. The aim of this study was to conduct an evaluation of sensibility and specificity of the advanced method Near infra-red transillumination system (NIR-TI), to confirm whether it could be useful in evaluating early approximal white spot lesions. Twenty-eight hemi tooth sections of different thickness (1.5 to 5mm) were examined, some of them with natural caries, and other sound. Images from NIR-TI and digitalized radiographs were compared by two trained and experienced examiners, to evaluate the presence or absence, and also the depth of those lesions. As a gold standard, the samples were sectioned and subjected to histological analysis using stereomicroscope and reflected light. Comparing results, a kappa test for question 1 and 2 was calculated: (0.11/0.27) and (0.47/0.51) for x ray and NIR-TI, respectively. The sensibility of each examiner were: 0.2 Ex1; 0.44 Ex2 on x ray; and 0.88 Ex1; 0.875 Ex2 on NIR TI; and the specificity 1.0 Ex1; 1.0 Ex2 on x ray; and 0.7 Ex1; 0.75 Ex2 on NIR TI. This study corroborated with the literature and clearly demonstrates that NIR transillumination system has considerable potential for the imaging of early approximal white spot lesions.

Descriptors: Dental caries; Transillumination; Digital Radiography

Introduction

The knowledge about the caries process has continued to advance, with the vast majority of evidence supporting a dynamic process which is affected by numerous modifiers tending to push the mineral equilibrium towards remineralisation and demineralisation [1]. Caries is a “continuum” rather than the macroscopic cavitation that is the late stage of the disease process [2], and many parameters estimate caries activity, such as salivary flow, sugar intake, oral hygiene, diet, number of new caries lesions and past caries experience [3].

Several methods of dental caries detection have been used for more than half a century. The main drawbacks of conventional methods, such as X ray, for detection of dental caries in oral diagnosis, are lesions located at proximal surfaces are not very often detected by radiographic examination. Thus, to confirm the diagnosis, one must still rely on the dentists subjective interpretation. Furthermore, it is difficult to evaluate a lesions progression, and that some clinicians decide on an unwarranted invasive intervention [4].

Sound enamel consists mainly of hydroxyapatite crystals which are very densely packed, giving the enamel a glass-like, translucent appearance. In the search for more accurate diagnostic approaches, investigators have used alternative non-invasive and instrument-based techniques based on variation in optical properties where incident light interacts with the tooth, by reflection, back-scattering or absorption [5] for detecting and quantifying demineralization of lesions [6,7]. Previous research by Jones et al. (2003) [8], showed the magnitude of scattering, at ~1300nm wavelengths is more than a factor of 30 times lower than in the visible range. This translates to a mean free path of 3.2mm for 1310nm photons [9].

All analyses used for the evaluation of diagnostic tests depend on a comparison with a "gold standard" or the "truth". Browner *et al.* (1988) [10] remind us that truth is only a matter of degree or acceptability, or, as Newhauser and Yin (1991) [11] say, "the best there is". Ability to measure incipient disease and even pre-disease susceptibility or high-risk states has caused us to re-examine what we actually mean by the words "disease" and "normal".

Focusing on development and validation of this new visual method of caries detection and diagnosis, described by Jones *et al.* (2003) [8] in which the magnitude of light scattering within the tooth decreases exponentially at longer wavelengths in the transillumination system, we decided to determine the sensibility and specificity of the near IR Transillumination analyzing natural white spots.

Material and Methods

We collected 14 human teeth extracted at the Human Teeth Bank of the Departament of Prostest and Dental-Facial Surgery of the Universidade Federal de Pernambuco, after the Ethics Committee approved the study (268/2007). Immediately all teeth were stored in a physiological saline solution 0,9% to preserve tissue hydration.

All teeth were properly identified, and has the crowns of fourteen teeth (premolares and molares) with approximal surfaces that had small white or brown spot lesions or any other signs of demineralization, but without cavitations were selected. Then, the samples had both the mesial and distal faces cut off, with a Low Speed diamond Whellsaw, model 650, SBT, inc. Fourteen plano-parallel sections of

various thickness 1,5 to 5mm were cut vestibulo-lingually paying attention to preserve regions of the proximal face which contained by white spots.

Based on the methodology described by Jones et al. (2003) [8], a similar system was built at the Optoelectronics and Photonics Laboratory at the Universidade Federal de Pernambuco, Brazil. The basic experimental scheme is illustrated in figure 1. We demonstrate the application of TI system with two different wavelengths: 1280nm SLD-571 (model SLD, SUPERLUM, Moscow, Russia) with an output power of 5mW and a bandwidth of 64,6nm; 1400nm Raman (Raman Fiber Laser, Key Optical System, China) with an output power of 50mW and a bandwidth of 0,6nm;

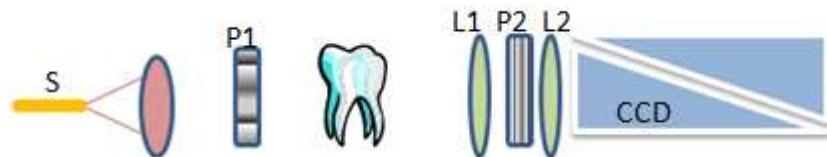


Figure 1: NIR Transillumination set up consists of a broadband light source (S), crossed linear polarizers (P1) and (P2), lenses (L1) and (L2), and the Micron Viewer IR camera (CCD).

Two crossed polarizers were placed perpendicular to each other to experimentally block out the ambient light from saturating the detector; a CCD camera with spectral response characteristic at 400 nm to over 2 μm (MicronViewer 7290A, Electrophysics, Fairfield, NJ, USA) was used. Due to the natural tooth contours, efforts were made to assure that the light travelled perpendicular to the analyzed sections, and a diffuser was used behind the sample to depolarize the illuminated light uniformly around the tooth. The 32-bit digital images were captured using Spiricon Laser Beam Diagnostics (LBA-PC, Version 2.5, Utah, USA) and

analyzed with a downloadable image processing program, ImageJ (NIH, Maryland, USA).

All teeth was submitted for comparison of the NIR transilluminations systems with Convencional X ray. The image was acquired using Ultra Ektaspeed Plus (Kodak) at an angle and distance that would simulated a clinical bitewing perpendicular of the sample. The radiograph Spectro II (Model 784782m Dabi Atlante, Sao Paulo) was adjusted at 15mA and 5 impulses. After the radiographic films were developed using fresh solutions (Revelator and Fixer, KODAK). All films were dried, and then digitalized by NIKON coolpix 4500 (4.1Mp) and negatoscopic illumination.

Both proximal surfaces of each tooth coronal to the cemento-enamel junction was included in this study, but analyzed separately as 28 samples. This was done intentionally to avoid the psychological effect that the assessment of one proximal surface could possibly bias the assessment of the neighboring surface. All images were qualitatively and quantitatively analyzed by two dentists specialists in radiology, and two questions with scale, the first correlates if the detection is clear, questionable, or unclear (Table 01). And the second, evaluate the lesion extension, by the scale indicated (Table 02).

Code	Question 01
01	probably no caries/dentine caries
02	questionable
03	probably caries/ dentine caries

Table 1: Qualitative question about caries diagnosis .

Code	Question 02
00	sound
01	enamel caries
02	caries reaching but not crossing the enamel-dentine junction
03	caries into dentine

Table 2: Quantitative questions eluciding the caries extension.

All images were presented on a Power Point Presentation and both examiners evaluated each sample separately without compares between x ray and NIR TI images. Figure 2 (a) and (c) shows two samples images by x ray, and (b) and (d) are images by NIR TI.

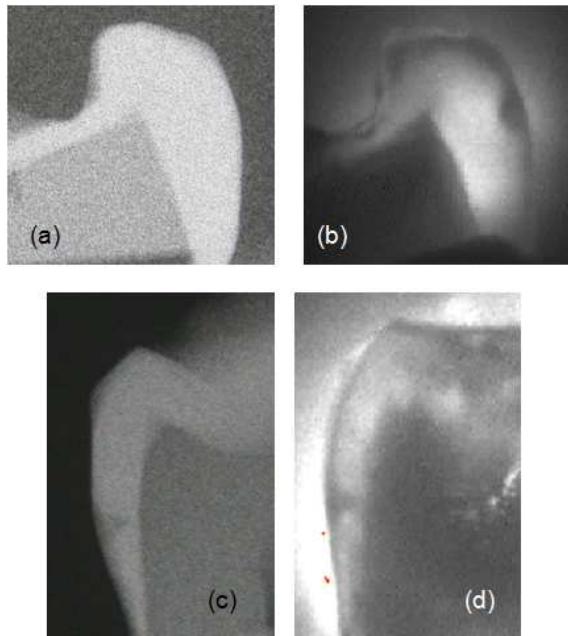


Figure 2: Evaluation of lesion caries contrast comparing images from both techniques. Images (a) and (b) from the same sample shows differences on contrast and detection of the lesion. In the other sample, images (c) and (d) are possible to detect the lesion in both techniques.

The samples were then sectioned in the middle, in the same first direction, using a diamond saw mounted in a microtome (LB 100 - Beltec). And an experienced examiner evaluated the two sections of each site under a stereomicroscope and reflected light, and the side with more extensive alterations was classified according the both tables 1 and 2.

The reproducibility of the evaluation system was assessed using unweighted kappa statistics. This was repeated being realized for repeated reading carried out by each examiner. Sensibility and specificity were calculated applying the McNemars Change test t to compare the performance of the diagnostic methods for each examiner after dichotomization of histological results.

Results

Table 1 gives unweighted kappa values for inter-examiner reproducibility for each ranked scale. The kappa test is a way to quantify the level of agreement by both examiners. Kappa statistic showed medium association for NIR TI technique, and low association for X Ray, even though all examiners were specialist and worked together as radiologists in the clinician settings.

Diagnostic Methods	Kappa test Question 01	Kappa test Question 02
NIR	0,47	0,51
X Ray	0,11	0,27

Table 3: Unweighted kappa values for inter-examiner reproducibility for ranked scoring systems for each of diagnostic methods.

The low association between both examiners is most likely a result of the fact that only a low of 28 samples were evaluated, of which 17 contained white spots and 11 were sound. Additionally, it is difficult to detect caries precoce using X ray.

Sensitivity and specificity tests are independent from disease prevalence because they are determined only from samples/patients which/who have disease (sensitivity) or only from samples/patients who do not have disease (specificity) [12].

Based on these parameters, only 28 samples were evaluated, which can interfere in our statistic Kappa test.

To make our discussion easier it's interesting to emphasize the meaning of sensitivity and specificity: *Sensitivity*, or the true-positive ratio, calculated as $TP/(TP+FN)$, measures the proportion of diseased patients correctly identified as positive. And *Specificity*, or the true-negative ratio, calculated as $TN/(FP+TN)$, measures the proportion of disease-free patients correctly identified as negative. So all observations detected were evaluated, looking for false negative and false positive results.

Sensitivities and specificities are shown in Table 2. NIR and X ray showed similar sensitivities and specificities ($p>0.05$) for both examiners. But the overall analysis, shows that NIR had a better performance than the X ray device, even though there was no lack of professional experience in using this NIR system.

Diagnostic Methods	Sensibility (%)		Specificity (%)	
	Ex 1	Ex 2	Ex 1	Ex 2
NIR	0,88	0,875	0,7	0,75
X Ray	0,2	0,44	1	1

Table 4: Performance of the diagnostic methods in diagnosing proximal white spot lesions using the table scale: sensibility and specificity.

Discussion

The dentist and patient are interested in knowing the probability that disease is present or absence when a diagnostic test is read as positive or negative. In the search for new technologies to identify very early stages of the disease process, it

will be even more important to show a direct relationship between the diagnostic test results and the patient outcome of interest. Due to the absence of effectiveness methods for approximal white spot is necessary to evaluate the sensibility and specificity of a new detection method of initial enamel caries disease it is necessary,

Film is a relatively inefficient radiation detector and requires relatively high radiation exposure [13]. The radiation exposure of dental x-ray is admittedly relative low compared to other diagnostic procedure but their high frequency, most often taken on a yearly basis, and risk associated with ionizing radiation exposure from artificial sources and from natural background emphasises the importance of avoiding exposure of patients to unnecessary irradiation [14]. In addition, the detection of early caries, as white spot lesions, is poorly showed by x ray images, due to the small variation of density on this lesions.

Our results show a good sensibility and specificity for NIR method detection of early caries due to the inability of light to pass through the tooth on the white spot region. White spot lesions are poorly shown/detected by x ray (observe low sensibility Ex1 0,2 and Ex2 0,44). However, the high specificity of the conventional x ray method can be explained, by the reduced chance to detect a false positive result, and consequently high specificity.

The literature shows that the new detection tools have been developed to identify those lesions that would be amenable to remineralising therapies [15]. In this context, it will be possible to get in a situation in which there is no true gold standard. However, a new diagnostic test can still become valuable if it used in addition to other measures. In situations where there are accepted diagnostic tests, a new

diagnostic test could still improve the diagnostic process by being less costly, more effective, less risky, or easier to conduct.

Conclusions

This study agrees with the literature and clearly demonstrates that NIR transillumination system has considerable potential for the imaging of early approximal white spot lesions. It was possible to evaluate the scientific theory of the test with our currents methods, and promote findings into clinically relevant measures of early caries disease that can be useful to the process of diagnosis and patient follow-up. In the future, NIR may prove to be a valuable technique in the biomedical field, and these researches are providing the science base for tomorrow.

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Characterization of enamel in deciduous teeth by Optical Coherence Tomography: *in vitro* comparison between 850nm and 1280nm

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Abstract

Caries is a disease that affects both deciduous and permanent teeth, therefore new caries diagnostic methods need to be tested on the deciduous teeth as well. This work reports the application of optical coherence tomography (OCT) to generate image of sound dental structure and with natural caries of *in vitro* human deciduous teeth. Images of enamel and dentin in deciduous teeth of thin thickness (~1.5mm) were obtained perpendicular to the enamel surface, by two OCT systems operating around 1280nm and 850nm, and compared with histology as the gold standard. The results demonstrate the efficacy of the OCT technique to measure, and characterize the enamel layer, proved statistically by test t-student presents p-value = 0.823. The technique presents great potential to be used on pediatric dentistry clinical, with no pain and early caries detection.

Keywords: Optical Coherence Tomography, Deciduous tooth, Enamel, Caries.

Introduction

The Optical Coherence Tomography (OCT) consists of a new imaging technique for diagnosis of transparent and semitransparent structure, that produces bi- or three-dimensional pictures, with less than $10\mu\text{m}$ spatial resolution [1], and has been widely used to study bio-tissues, as reviewed in [2]. In Dentistry, the first applications were reported in 1998 [3], and since then it has been used for diverse purposes, as characterization of periodontal structures [4,5], recurrent caries's detection and marginal adaptation of restorations [6] and precocious detection of oral cancer [7]. More recently, besides evaluation of enamel interface restoration [6], also early caries diagnostics [8], and the analysis of the performance of the dental materials [9,10] have been studied and reported by our group.

The key elements of an OCT setup include a broadband light source, whose spectral width limits the axial spatial resolution and penetration; an interferometer, which generally employs a Michelson design containing in one of the arms the sample and in the other arm a delay line and an optical detector, whose signal output is electronically treated and fed to a computer for the image generation. Additionally, most OCT techniques described for imaging dental tissue have used Super Luminescent Diodes, as the light source with wavelengths of 840 to 1310 nm [1,11], since they can provide spectral bandwidth wide enough to support axial resolution $\sim 10\mu\text{m}$ with very good beam quality, besides being much more economical than use a femtosecond laser source.

Two domains can be exploited for implementation of an OCT system: the time domain (TD-OCT) or the spectral domain (SD-OCT). In the time domain, the optical delay line arm basically consists of either a movable arm or a Fourier domain delay

line [12]. In the spectral domain, there are no movable parts in the interferometer arms (except for lateral displacement of the beam on the sample), and the recombined beams from the interferometer are sent to a spectrometer and is Fourier analyzed. It has been shown that SD-OCT has several advantages over TD-OCT, including sensitivity and fast acquisition data, and since the first report on imaging implementation using SD-OCT [13] its use has been widespread.

As any optical caries diagnosis technique, it is necessary to understand dental hard tissue optics properties inherent to the complex nonhomogeneous biological structures. Dental enamel is an ordered array of inorganic apatite-like crystals surrounded by a protein/lipid/water matrix [14]. The crystals are clustered together and roughly perpendicular to the tooth surface, due to the scattering distributions are generally anisotropic and depend on tissue orientation relative to the irradiating light source [15, 16, 17] in addition to the polarization of the incident light.

The near-IR region from 780 to 1550nm offers the greatest potential for new optical imaging modalities due to the weak scattering and absorption in dental hard tissue [18]. The magnitude of light scattering in dental enamel is expected to decrease following a $1/\lambda^4$ law, λ being the source wavelength, due to the size of the principal scatters in enamel [14]. By analyzing the optical properties of carious enamel one can establish a method of characterizing the severity of the carious lesion. The first sign of visual appearance is the white spot lesions that increases the backscattering due to the higher porosity of the demineralized region [19, 20, 21].

The success of the dental treatment depends on the integral knowledge of the properties, structure and function of dental structures, which constitutes the necessary biological basis for taking clinical decisions as by the early detect of white spot lesion without pain, professionals can promote early fluoride therapy and during

remineralization, pores and tubules are filled with minerals, and those areas became more transparent.

Despite the differences in structure and composition of permanent and primary teeth, research about early caries detection by optical systems to pediatric dentistry clinical are still incipient worldwide. And as caries progresses more rapidly in deciduous enamel than in permanent enamel, new caries diagnostic methods need to be tested on the deciduous teeth as well [22]. Optical coherence Tomography (OCT) seem promising for the quantification of mineral loss from dental caries but have only been more thoroughly tested on the permanent dentition.

The objective of this study was to show a characterization of enamel layer structure, as well as detect and quantify mineral loss from natural carious lesions, using two different wavelengths in the near-infrared. The studies were also extended to the dentine.

Materials and Methods

The experimental study was carried out after approval by the ethical committee (268/2007) in accordance with the ethical guidelines in research with human participants by the Center of Health Sciences, Universidade Federal de Pernambuco, Brazil.

In these research four incisors and two canines primary teeth, lost physiologically, were used as samples. The teeth were available at the Tooth Bank of the Universidade Federal de Pernambuco and stored in physiological saline 0,9%. All

of them were transversally cut resulting in three sections (~1,5mm), by the Low Speed diamond Whellsaw, model 650, SBT, inc., with irrigation.

To perform OCT imaging of teeth, we used two home built OCT systems, whose schematic diagrams are shown in figure 1. Figure 1(a) shows the schematic of the SD-OCT system operating at the wavelength of 850nm. The broadband source is a superluminescent diode (Broadband SLD Lightsource S840, SUPERLUM, Moscow, Russia) delivering up to 25mW and with a 49,9nm bandwidth, which gives an axial resolution of 6 μ m. After traveling through the all-fiber beam splitter, the reflected beams from the sample and mirror are recombined and sent through a purpose designed spectrometer consisting of a lens collimator system, 1200 l/mm grating and CCD (ATMEL, 2048 pixels, 12 bits, California - United States). The maximum incident power on the sample was approximately 5mW. The output is sent to a personal computer with a LabView based imaging program.

Figure 1(b) shows the schematic of the TD-OCT system operating at the central wavelength of 1280 nm, maximum average power 5mW, delivered by a superluminescent diode (model SLD-571, SUPERLUM, Moscow, Russia), with a 64.6 nm bandwidth, which represents an axial resolution of 11 μ m. As with the system in figure 1(a), an all-fiber beam splitter is used, but in this case the delay line is a Fourier domain delay line [12] consisting of a grating and a scanning galvo. The recombined beams are fed into a photodetector and associated electronics, and the output is sent to a personal computer with a LabView based imaging program.

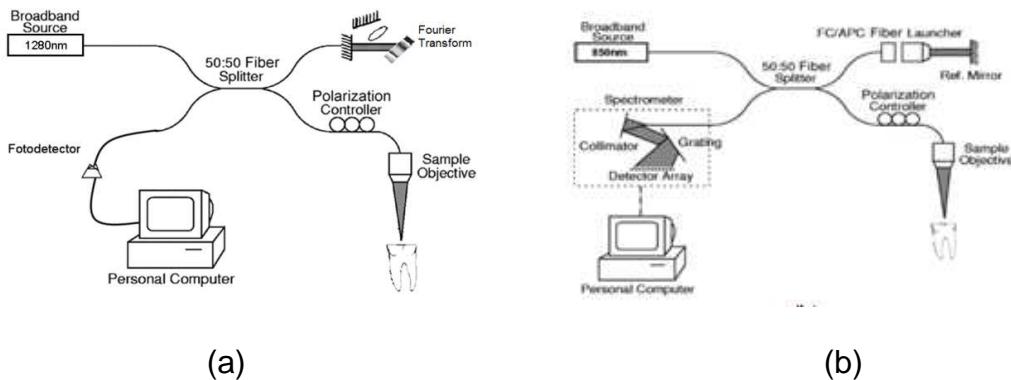


Figure 1: Schematic diagram of the optical coherence tomography system.
 (a) SD-OCT operating at 850nm (b) TD-OCT operating at 1280nm with
 a Fourier domain delay line.

The images of the enamel thickness were taken by scanning the enamel surface in all four faces (vestibule, lingual, mesial and distal). The laser penetrated into the tooth structure and a tomographic image of the frame, perpendicular to the axis of tooth, was obtained. After the image construction by OCT (1280nm and 850nm), all samples were compared with histological images from both sides of the thickness by an optical microscope with a CCD adapted.

All images were analyzed with a downloadable image processing program, ImageJ (NIH, Maryland, USA). The enamel thickness was measured in both techniques and values were compared by correlation statistics test. Based in the structure of sound enamel, it was easily observed small alterations of white spot demineralised, confirmed by histological evaluation.

Results

For comparison purposes, we first show in figures 2 (a), (b) and (c) images, obtained by optical microscope, of three sections: cervical, medial and incisal, respectively, of one of the studied samples. The dimensions of enamel layer around the tooth are shown by the scales.



Figure 2: Optical Microscope image of the three thickness of one deciduous tooth.

(a) incisal, (b) medial and (c) cervical sections.

In order to obtain more accurate OCT images, the sections were cut before obtaining the images by OCT and all sections were measured from both sides. The values of enamel layer ranged from 150 μ m to 1000 μ m on different faces of the tooth, as expected.

Figure 3 shows a comparison of the OCT images with the optical microscope visualization. It can be clearly identified with precision the enamel layer (E), the enamel-dentin junction (EDJ) and a thin layer of dentin (D) of all samples examined in both microscopy and OCT images. The structures in the OCT images are distinguished due to the different gray levels (or blue levels, in the case of the 850nm system), where the limits of enamel layers appears whiter (highest scattered intensities) and the dentin with the darker level (lowest scattered intensity).

In figures 3(d), (e) and (f), it is shown a layout of OCT images, which were constructed by superimposing OCT scans at different faces of the tooth section. It is possible, from figures 3(e) and (f) to observe the enamel structures (superficial layer) that were clearly delineated due to the strong birefringence, while the dentin, due to the anisotropic light propagation through dentinal tubules, which backscatters light in very different ways, makes a diffuse image.

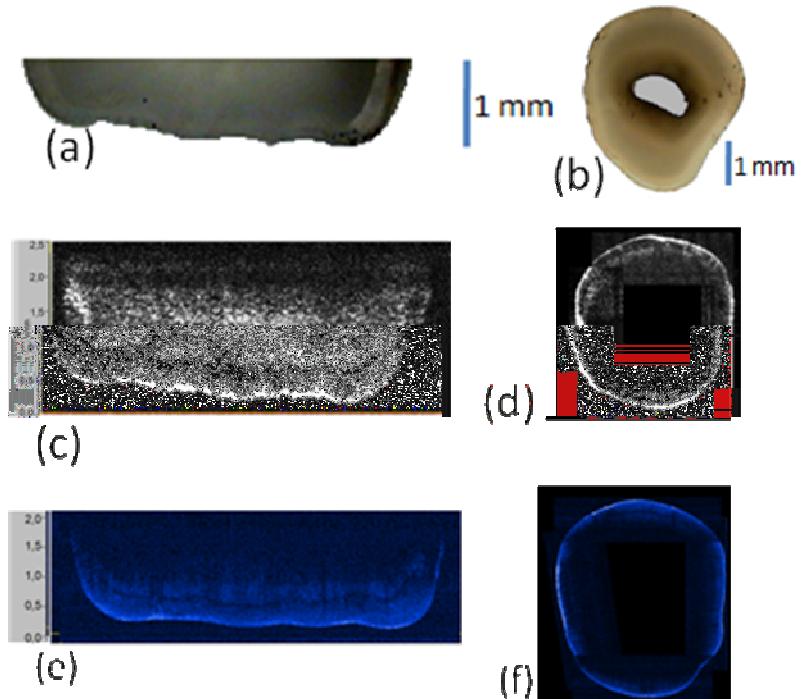


Figure 3: (a) Lingual face of a deciduous sample by histological image; (b) OCT image at 1300nm and (c) OCT image at 840nm. Figure 3 (d) histological, (e) layout OCT 1300nm and (f) layout OCT 840nm. The OCT images were constructed by superimposing OCT scans at different faces of the tooth section.

Notice that the measurements in the OCT value shown on the scale must be divided by the refraction index of enamel ~1,62 [3]. Analyzing the same image by both OCT systems, it is possible to see a better resolution from 840nm due to the spectral domain, but a deeper penetration of light on OCT of 1300nm.

In order to confirm the precision of each OCT system measurements by statistical tests, we carried out a series of measurements, in total of $n=72$ for each image systems, evaluated by ImageJ Software.

Table 1 shows the results, comparing $n=72$ samples, by minimum and maximum of the enamel layer obtained by all image systems. The mean and standard deviation behaved in the same way, what was corroborated by statistical

tests, as t-student and correlation test, indicating that both OCT Systems were calibrated and efficient to examine enamel layer with precision.

Image System	n	Min	Max	Mean	SD	IC (95%)
OCT 840nm	72	0.130	0.875	0.388	0.174	0.347-0.429
OCT 1300nm	72	0.137	0.984	0.406	0.188	0.362-0.451
Histology	72	0.130	0.979	0.396	0.175	0.355-0.437

Table 1: Comparison of the layer measurements by the three image systems.

(n) number of samples, Min (minimum value), Max (maximum value), Mean (mean value), standard deviation and IC (correlation index).

Test t-student presents p-value = 0.823.

The OCT clearly demonstrates the capacity of quantitative assessment and penetration of the radiation on the enamel and dentin of deciduous tooth, compared with the microscope. The deeper penetration depth of the light at 1280nm compared to 850nm is due to a remarkable reduction of absorption and scattering coefficients of the enamel and dentin at 1280nm [23].

As a final example, figure 4 shows a superposition of the four faces of one section of one tooth samples, but differently from figure 3, the tooth presents caries decay. In figure 4(a), the circle indicates the lesioned region, which is identified by the increased scattering. By using the imageJ program, the contrast between the lesioned and healthy enamel is measured, as shown in figure 4(b). In the gray scale, represented in the Y-axis in arbitrary units, the healthy enamel is the background with values below 0.05, whereas in the lesioned region the values approach 0.15. Therefore, it is possible to determine, through contrast value, an increase in

backscattered intensity of the order of 2-3, which corroborates early results by Huynh et al. (2004) using polarization sensitive OCT.

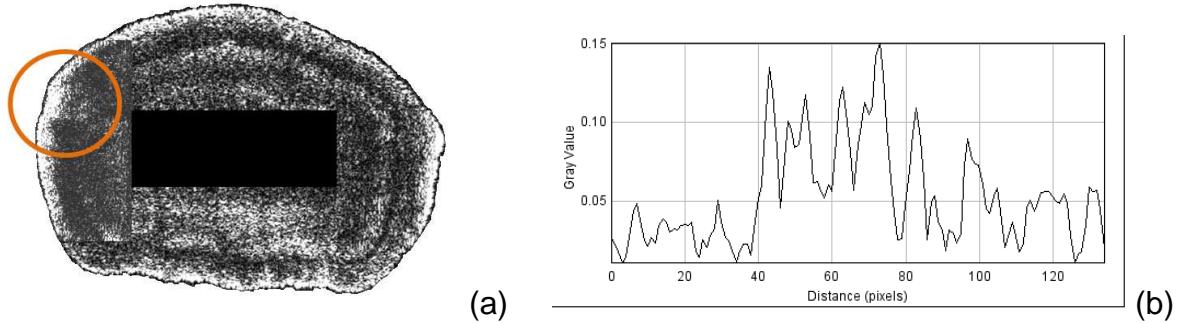


Figure 4: Evaluation of contrast on the caries decay region, observe in graphic plot the increase of backscatter intensity in order of 2-3.

Discussion

Early caries can be remineralized with therapeutic agent and/or improved oral hygiene if detected at an incipient stage [24]. However, the effectiveness of these preventive measures can only be determined with a method that can quantitatively monitor the changes overtime in the mineral status of the caries [25].

A full characterization of the enamel surface is extremely important in caries diagnosis, since early lesions can be appropriately detected. OCT has already been proven to be a feasible method for clinical use and detection of early caries. Even the lesion severity can be determined with a conventional OCT system by measuring the loss of penetration depth caused by increased attenuation due to demineralization [26]. This is particularly important to implement in the proximal surfaces due to uniform optical penetration and surface reflectivity.

The caries evolution in deciduous teeth is faster than in permanent ones, and therefore a fast and non-invasive evaluation is imperative. Also, as the enamel

thickness is smaller than in permanent teeth, a system with axial resolution of the order of $\sim 10\mu\text{m}$ would be advantageous.

Our two OCT systems operated with near infrared (NIR) light, notably 1310nm and 840nm, in which the first one improves the axial imaging penetration depth over wavelengths in the visible range, whereas the second one, due to the optical source used, presents a higher axial resolution. It is worth noting that, given a source at 1300nm with the same – or better – axial resolution than the one we used, this would definitely be the preferred source. The obtained results, when compared with the histology, confirmed the OCT ability to early caries detection, and in this case applied to deciduous teeth.

Conclusions

In accordance with the results presented for the OCT's with wavelength of 1280nm and 850nm, the two techniques were effective to early caries identification in deciduous teeth. Furthermore, our experiment corroborated the deeper penetration depth for 1280nm OCT.

OCT possesses great potential to be used routinely in clinical practice for the complex diagnosis of early enamel caries, promoting possible remineralized preventive measures.

Acknowledgements

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Registro do SISNEP FR – 150899

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Registro CEP/CCS/UFPE Nº 276/07

Titulo: "Avaliação de Técnicas Diagnósticas de Lesões de Cárie Interproximal: Raio X, Transiluminação a 1300nm e Tomografia por Coerência Óptica "

Pesquisador Responsável: Ana Marly Araújo Maia

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Ressaltamos que o pesquisador responsável deverá apresentar relatório anual da pesquisa.

Atenciosamente

Prof. Geraldo Bosco Lindoso Couto
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A

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Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance.

Relevant Documents: Sample Manuscript, Exclusive Licence form

Useful Websites: Submission Site, Articles published in International Journal of Paediatric Dentistry, Author Services, Blackwell Publishing's Ethical Guidelines, Guidelines for Figures.

1. GENERAL

International Journal of Paediatric Dentistry publishes papers on all aspects of paediatric dentistry including: growth and development, behaviour management, prevention, restorative treatment and issue relating to medically compromised children or those with disabilities. This peer-reviewed journal features scientific articles, reviews, clinical techniques, brief clinical reports, short communications and abstracts of current paediatric dental research. Analytical studies with a scientific novelty value are preferred to descriptive studies.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after acceptance of a manuscript for publication in *International Journal of Paediatric Dentistry*. Authors are encouraged to visit Blackwell Publishing Author Services for further information on the preparation and submission of articles and figures.

In June 2007 the Editors gave a presentation on How to write a successful paper for the *International Journal of Paediatric Dentistry*.

2. ETHICAL GUIDELINES

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4. MANUSCRIPT TYPES ACCEPTED

Original Articles: Divided into: Summary, Introduction, Material and methods, Results, Discussion, Bullet points, Acknowledgements, References, Figure legends, Tables and Figures arranged in this order. The summary should be structured using the following subheadings: Background, Hypothesis or Aim, Design, Results, and Conclusions and should be less than 200 words. A brief description, in bullet form, should be included at the end of the paper and should describe What this paper adds and Why this paper is important to paediatric dentists.

Review Articles: may be invited by the Editor.

Short Communications: should contain important, new, definitive information of sufficient significance to warrant publication. They should not be divided into different parts and summaries are not required.

Clinical Techniques: This type of publication is best suited to describe significant improvements in clinical practice such as introduction of new technology or practical approaches to recognised clinical challenges.

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5. MANUSCRIPT FORMAT AND STRUCTURE

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The whole manuscript should be double-spaced, paginated, and submitted in correct English. The beginning of each paragraph should be properly marked with an indent.

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Summary should be structured using the following subheadings: Background, Hypothesis or Aim, Design, Results, and Conclusions.

Introduction should be brief and end with a statement of the aim of the study or hypotheses tested. Describe and cite only the most relevant earlier studies. Avoid presentation of an extensive review of the field.

Material and methods should be clearly described and provide enough detail so that the observations can be critically evaluated and, if necessary repeated. Use section subheadings in a logical order to title each category or method. Use this order also in the results section. Authors should have considered the ethical aspects of their research and should ensure that the project was approved by an appropriate ethical committee, which should be stated. Type of statistical analysis must be described clearly and carefully.

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(ii) **Clinical trials** should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist should also be included in the submission material.

International Journal of Paediatric Dentistry encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, <http://clinicaltrials-dev.ifpma.org/>, <http://isrctn.org/>. The clinical trial registration number and name of the trial register will then be published with the paper.

(iii) **DNA Sequences and Crystallographic Structure Determinations:** Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

Results should clearly and concisely report the findings, and division using subheadings is encouraged. Double documentation of data in text, tables or figures is not acceptable. Tables and figures should not include data that can be given in the text in one or two sentences.

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Bullet Points should include two headings:

- *What this paper adds and
- *Why this paper is important to paediatric dentists.
- *Provide maximum 3 bullets per heading.

Review Articles: may be invited by the Editor. Review articles for the *International Journal of Paediatric Dentistry* should include: a) description of search strategy of relevant literature (search terms and databases), b) inclusion criteria (language, type of studies i.e. randomized controlled trial or other, duration of studies and chosen endpoints, c) evaluation of papers and level of evidence. For examples see:

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Clinical Techniques: This type of publication is best suited to describe significant improvements in clinical practice such as introduction of new technology or practical approaches to recognised clinical challenges. They should conform to highest scientific and clinical practice standards.

Short Communications: Brief scientific articles or short case reports may be submitted, which should be no longer than 3 pages of double spaced text. They should contain important, new, definitive information of sufficient significance to warrant publication. They should not be divided into different parts and summaries are not required.

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A maximum of 30 references should be numbered consecutively in the order in which they appear in the text (Vancouver System). They should be identified in the text by bracketed Arabic numbers and listed at the end of the paper in numerical order. Identify references in text, tables and legends. Check and ensure that all listed references are cited in the text. Non-refereed material and, if possible, non-English publications should be avoided. Congress abstracts, unaccepted papers, unpublished observations, and personal communications may not be placed in the reference list. References to unpublished findings and to personal communication (provided that explicit consent has been given by the sources) may be inserted in parenthesis in the text. Journal and book references should be set out as in the following examples:

1. Kronfol NM. Perspectives on the health care system of the United Arab Emirates. *East Mediter Health J.* 1999; 5: 149-167.
2. Ministry of Health, Department of Planning. Annual Statistical Report. Abu Dhabi: Ministry of Health, 2001.
3. Al-Mughery AS, Attwood D, Blinkhorn A. Dental health of 5-year-old children in Abu Dhabi, United Arab Emirates. *Community Dent Oral Epidemiol* 1991; 19: 308-309.
4. Al-Hosani E, Rugg-Gunn A. Combination of low parental educational attainment and high parental income related to high caries experience in preschool children in Abu Dhabi. *Community Dent Oral Epidemiol* 1998; 26: 31-36.

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