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RADIOGRAFIA DIGITAL E A TÉCNICA DE SUBTRAÇÃO NO MONITORAMENTO DA DESMINERALIZAÇÃO E REMINERALIZAÇÃO DO ESMALTE DENTÁRIO

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de doutora em Radiologia Odontológica.

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"Nada na vida deve ser temido, somente compreendido".

Marie Curie, Física

"Quando nada é certo, tudo é possível".

Margaret Drabble, escritora

RESUMO

O objetivo deste estudo *in vitro* foi comparar dois protocolos de remineralização em lesões de cárie no esmalte dentário, avaliados por meio de análises de cálcio (Ca) e fósforo (P_i), dureza do esmalte, microscopia de luz polarizada e subtração radiográfica digital (SRD); avaliar a viabilidade da utilização de dois diferentes sistemas de radiografias digitais, placa PSP (photostimulable storage phosphor) e sensor CMOS (complementary metal oxide semicondutor), no diagnóstico de desmineralizações, e a acurácia das radiografias digitais convencionais (RDC) e três métodos de SRD (linear, avançada e logarítmica) no diagnóstico de mudanças minerais; e comparar o ruído e reprodutibilidade das imagens de SRD lineares e logarítmicas produzidas a partir de dois sistemas de radiografias digitais. Para isso, lesões de cárie artificiais foram criadas em 100 superfícies proximais de dentes hígidos. Vinte dentes foram mantidos como controle e oitenta foram submetidos a dois diferentes protocolos de remineralização em 4 e 8 semanas, com a contínua imersão em saliva artificial e um tratamento adicional com flúor. Radiografias digitais foram realizadas antes e depois dos protocolos de remineralização. Cinco examinadores avaliaram a desmineralização e as mudanças minerais nas RDC, dispostas lado a lado, e três métodos da SRD. As análises de Ca / P_i e a colocação dos dentes na solução remineralizante foram considerados como padrão ouro. A média dos tons de cinza e o desvio padrão (DP) no histograma foram também mensurados. As concentrações de Ca e P_i na saliva artificial após os tratamentos foram significativamente menores do que a solução original (p<0,05); e por meio da SRD foi possível verificar diferenças entre as imagens. Entretanto, o teste de dureza e a microscopia de luz polarizada não detectaram nenhuma alteração. O sistema CMOS foi significativamente mais acurado do que a sistema PSP no diagnóstico da desmineralização e mudanças minerais, assim como a SRD linear no diagnóstico de mudanças minerais. Também foram estatisticamente diferentes os valores da média dos níveis de cinza e do DP entre os dois sistemas. Foi concluído que (a) o tratamento adicional de flúor promoveu valores maiores de ganho mineral; (b) a análise de Ca / P_i na saliva artificial foi o método mais sensível na avaliação de alteração mineral; (c) a imagem de SRD linear é um método válido na detecção do aumento de intensidade, como sinal de ganho mineral; e (d) as imagens de SRD utilizando as placas PSP tiveram um menor ruído do que nas imagens geradas pelo sensor CMOS.

Palavras-chave: Cárie dentária, Flúor, Análise química, Ruído, Radiologia, Intensificação de imagem radiográfica.

ABSTRACT

The aim of this *in vitro* study was to compare two remineralization protocols of artificial carious lesions in enamel, evaluated by Calcium (Ca) and Phosphorus (P_i) analysis, cross-section hardness test, polarized light microscopy and digital subtraction images (DSR); to assess the feasibility of using two different systems of digital radiography, photostimulable storage phosphor (PSP) plate and complementary metal oxide semiconductor (CMOS) sensor on the demineralization diagnosis, and the accuracy of digital conventional radiographs (DCR) and three methods of DSI (linear, advanced and logarithmic) on mineral changes diagnosis; and, to compare noise and reproducibility in linear and logarithmic DSI produced from two digital radiography systems. Artificial caries-like lesions on 100 approximal surfaces of sound teeth were produced. Twenty teeth were kept as control and eighty teeth were subjected to two different remineralization protocols for 4 and 8 weeks, with continuous immersion in artificial saliva, and additional fluoride treatment. Digital radiographs were taken before and after the remineralization protocols. Five examiners assessed demineralization and mineral changes on DCR, placed side by side, and three methods of DSI. Ca / P_i analysis and the placement of the teeth on the remineralization solution was the gold standard. The mean shades of gray and the standard deviation (SD) of the histogram were also assessed. The concentrations of Ca and P_i in the artificial saliva after the treatments were significantly lower than the original solution (p<0.05); and DSR showed differences between the images. However, the hardness test and polarized light microscopy did not detect any changes. CMOS system was significantly more accurate than PSP system on demineralization and mineral changes diagnosis, and also linear DSR on mineral changes diagnosis. It was also statistically significant different the values of mean shades of gray and SD between both systems. It was concluded that (a) the additional Fluoride treatment provided higher values of mineral gained; (b) Ca / P_i analysis in the artificial saliva were the most sensitive method of mineral change evaluation; (c) linear DSI is a valuable method to disclose an intensity increase, as a sign of mineral gained; and (d) DSR images created from PSP plates had less noise than images produced from CMOS sensor.

Key Words: Dental caries, Fluoride, Chemical analysis, Noise, Radiology, Radiographic image enhancement.

SUMÁRIO

INTRODUÇÃO

O início da lesão de cárie está associado com a desmineralização do esmalte dentário. A superfície do esmalte perde íons de cálcio (Ca) e fósforo (P_i) resultando na formação de uma lesão sem cavitação. Neste estágio, a lesão de cárie é reversível via processos de remineralização envolvendo a difusão destes íons na superfície restaurando as estruturas perdidas. Este processo de remineralização usando soluções de Ca e P_i ou um tratamento adicional com flúor (F) já foi estabelecido (Ingram & Edgar, 1994). Esse processo de reversão da lesão de cárie pode também ocorrer em altos níveis de perda mineral inicial, onde já tenha sido considerado que o processo de cárie "passou do ponto de retorno" (ten Cate, 2001; Mukai & ten Cate, 2002).

Um grande número de métodos tem sido utilizado na mensuração de mudanças que ocorrem nos tecidos dentários, incluindo testes que avaliam mudanças quantitativas das propriedades físicas, como o teste de dureza do esmalte seccionado longitudinalmente, mudanças na composição mineral (dosagens bioquímicas) ou por meio da microscopia de luz polarizada (Argenta *et al.*, 2003; Ganss *et al.*, 2005). Entretanto, estes métodos são utilizados em estudos *in vitro* e *in situ*, sendo necessário o estudo de métodos que possam quantificar a progressão (desmineralização) ou regressão (remineralização) das lesões de cáries em esmalte *in vivo*. Algumas técnicas como medida de resistência elétrica (Wang *et al.*, 2005), fluorescência quantitativa induzida por luminosidade (Pretty *et al.*, 2003) e fluorescência a laser (Lussi *et al.*, 2001), têm sido desenvolvidas com este propósito de mensurar as mudanças minerais em esmalte humano. Contudo, ainda não existe um método aceito, principalmente para o monitoramento de lesões de cárie interproximais.

Para o diagnóstico da cárie dentária, o exame radiográfico tem sido apontado como um método ideal (Gröndahl *et al.*, 1982; Espelid & Tveit, 1984; Pitts & Renson, 1986; Heaven *et al.*, 1990; Wenzel, 1995; Wenzel, 2000; Wenzel *et al.*, 2000), sendo que no que se concerne às superfícies proximais, a radiografia constitui-se em um procedimento essencial ao diagnóstico (Gröndahl, 1979). Além disso, com o monitoramento por meio de radiografias podemos verificar a progressão ou a paralisação de lesões cariosas. Porém, para se determinar pequenas alterações minerais que possam ocorrer durante um determinado período de tempo, são necessários métodos com alto grau de precisão, para que as mesmas possam ser mensuradas por um ou vários observadores sem grandes variações.

Embora o exame radiográfico seja um método sensível para o registro da perda mineral em esmalte e dentina, a interpretação correta das características radiográficas pode ser uma tarefa difícil, pois a extensão das lesões cariosas pode ser subestimada ou superestimada nas radiografias convencionais, em comparação com os achados clínicos e histológicos (Espelid & Tveit, 1984; Syriopoulos *et al.* 2000). Esta informação reforça a significância de se desenvolver técnicas inovadoras e mais precisas para o diagnóstico da cárie, adequando o plano de tratamento à severidade da lesão.

Basicamente, os erros de posicionamento do filme e do cabeçote de raios X são as principais fontes de distorção geométrica, que geralmente ocorrem juntos. Porém, esses erros podem ser minimizados através do uso de posicionadores e registros de mordida com material de moldagem (Hausmann *et al.,* 1996). Foram desenvolvidos e estão sendo usados também, meios de manipulação da imagem onde, através de uma matriz de transformação algorítmica, pequenas diferenças geométricas de exposição podem ser ajustadas (Webber *et al.,* 1984; Fisher *et al.,* 1994).

Além disso, mesmo com um perfeito alinhamento da projeção geométrica, fatores como radiação espalhada e o processamento radiográfico alteram a densidade e o contraste entre as duas radiografias. Uma variedade de métodos tem sido utilizada para que esta correção do contraste e densidade seja feita, com o cuidado de não remover informações de ganho ou perda de tecido mineral (Ruittmann *et al*., 1986; Likar & Pernus, 1997).

Os novos sistemas de radiografia digital com aumento da resolução da imagem e os recursos de manipulação, como ampliação e alteração do contraste e densidade, podem ser validados como uma alternativa para aumentar a acurácia no diagnóstico de cárie. Estes sistemas, comparados com radiografias convencionais proporcionam uma acurácia de diagnóstico semelhante (Wenzel 2006). Por sua vez, recursos tecnológicos como a subtração radiográfica digital figura como uma técnica que possibilita a detecção de alterações tênues nas estruturas mineralizadas da boca (Gröndahl *et al.*, 1983; Halse *et al.*, 1990; Maggio *et al.*, 1990; Wenzel & Halse, 1992; Wenzel *et al.*, 1993; Minah *et al.*, 1998; Wenzel, 1998; Ferreira *et al.*, 1999; Eberhard *et al.*, 2000; Wenzel *et al.*, 2000).

A subtração radiográfica digital (SRD) tem sido apontada como uma eficiente estratégia de processamento de imagens para a detecção de pequenas mudanças em tecidos duros e também pode se tornar adequada para o uso na cariologia (Wenzel *et al.*, 2000). No diagnóstico de desmineralizações interproximais ou oclusais, a SRD obteve valores maiores de acurácia e sensibilidade (Haiter-Neto *et al.*, 2005; Ricketts *et al.*, 2007). Um aumento de intensidade em áreas de desmineralizações oclusais e interproximais depois do uso de soluções de F também foi diagnosticado pela SRD (Halse *et al.*, 1990; Wenzel & Halse. 1992), mas ainda não existe uma comparação de diferentes métodos de SRD no diagnóstico de mudanças minerais. Também existem algumas pesquisas que foram desenvolvidas com o objetivo de avaliar as técnicas de subtração radiográfica digital como auxiliar para o diagnóstico e proservação das lesões de cárie (Gröndahl *et al.*, 1982; Halse *et al.*, 1990; Maggio *et al.*, 1990; Nummikoski *et al.*, 1992; Wenzel & Halse, 1992; Halse *et al.*, 1994; Sousa *et al.*, 1997; Minah *et al.*, 1998; Eberhard *et al.*, 2000). No entanto, poucos estudos abordam o monitoramento da progressão de lesões iniciais em esmalte.

A SRD foi introduzida na Odontologia nos anos oitenta (Webber *et al.*, 1982; Grondahl *et al.*, 1983) com o objetivo de comparar duas radiografias padronizadas feitas em um intervalo de tempo. Esta técnica consiste em subtrair as estruturas que não se alteraram entre os dois exames radiográficos resultando em uma imagem envolta por um fundo cinza neutro. Áreas de perda de tecido mineral são convencionalmente mostradas por um cinza escuro enquanto áreas de ganho aparecem como um cinza claro.

Em linguagem computacional, as imagens radiográficas são constituídas por pixels. O número de degraus ou níveis de cinza é determinado por 2^N, onde N é o número de bits em cada pixel. A representação digital de densidade em cada pixel é mais comumente representada por 8 bits, onde as numerações variam de 0 a 256, sendo o número 0 a cor preta e o 256 a cor branca (Balter, 1993). Na subtração radiográfica digital, subtraindo dois pixels iguais resultaria no numero 0, o que daria uma imagem preta. Para que isso não ocorra, o sistema automaticamente adiciona a cada subtração, pixel a pixel, o valor 128, o que resulta em uma imagem com um tom de cinza médio. As áreas em que a subtração dos pixels não for 0, o valor pode ser acima de 128, resultando em uma área mais clara, ou abaixo de 128, resultando em uma área mais escura.

A acurácia da técnica da subtração radiográfica digital em revelar alterações quantitativas de densidade óssea está na dependência da produção de radiografias padronizadas geometricamente, além de contraste e densidade semelhantes. Qualquer alteração entre a radiografia inicial e final na mesma região anatômica irá produzir áreas na imagem de subtração com um aumento ou diminuição da densidade, o que pode ser interpretado erroneamente como regiões de ganho ou perda mineral (Benn, 1990).

Teoricamente, o resultado da SRD são imagens que destacam as diferenças que ocorreram no período de tempo avaliado sobre um fundo relativamente uniforme. Entretanto, imagens de subtração podem conter uma variação dos níveis de cinza o qual é independente de mudanças produzidas por reais diferenças entre as duas imagens subtraídas. Estas diferenças "acidentais" podem interferir no diagnóstico e são chamados de ruído. Deve ser possível em subtração digital quantitativa detectar qualquer mudança nos valores dos pixels os quais realmente se originaram de mudanças no objeto através da eliminação da variação na sensibilidade dos pixels (ruído) (Yoshioka *et al*., 1997). Este ruído pode ser quantificado usando o desvio padrão (DP) do histograma, definindo a distribuição dos tons de cinza na imagem de subtração (Eraso *et al*., 2007). Quanto maior a quantidade de tons de cinza no histograma, mais ruído na imagem de subtração e maior será o DP (Haiter-Neto & Wenzel, 2005).

Na prática clínica, a utilização de meios de padronização geométrica como, por exemplo, registros de mordida bem como os meios de correção por meio de sistemas computadorizados são importantes. Além disso, uma correção do contraste e densidade entre as duas radiografias deve ser feita, pois variações no tempo de exposição e tipo de processamento podem contribuir, também, para uma falta de acurácia da técnica (Jassen *et al.,* 1989; Filder *et al*., 2000).

Um dos programas de computador utilizados para criar imagens de subtração radiográfica digital é o EMAGO® (Oral Diagnostic Systems, Louwesweg, Amsterdam, Holanda). Neste programa, dois comandos ("gamma correction" e "reconstruction") foram criados para minimizar estas condições indesejadas e viabilizar o uso da subtração radiográfica digital como método diagnóstico.

O comando "gamma correction" é utilizado para a realização de uma correção do contraste e densidade entre as duas radiografias, objetivando a uniformidade dessas duas imagens. Como já foi dito, a diferença na distribuição dos tons de cinza das radiografias pode interferir na detecção de pequenas alterações, gerando um ruído nas imagens, ou seja, produzindo diferenças entre as imagens que não sejam reais, dificultando o correto diagnóstico.

O comando "reconstruction" é utilizado para que as duas radiografias tenham a projeção geométrica mais semelhante possível. A reconstrução envolve o mapeamento da informação contida em uma imagem sobre o plano de projeção da radiografia inicial. Neste programa a reconstrução é feita por meio da marcação de quatro pontos posicionados no mesmo local nas duas radiografias, onde automaticamente é gerada a imagem reconstruída. Estes pontos geralmente são marcados próximos da área de interesse, sendo que se houver mais de uma área a ser avaliada, recomenda-se a criação de uma imagem de reconstrução para cada uma das áreas a serem analisadas.

Em 1998, Byrd *et al.* avaliaram a acurácia do alinhamento através de pontos, comparando três e quatro pontos, para minimizar discrepâncias geométricas, utilizando "chips ósseos" colocados em mandíbulas maceradas. Também, correlacionaram os dados em um estudo *in vivo.* Os resultados indicaram que o alinhamento através de quatro pontos pode aumentar a eficácia da SRD.

Existem dois tipos de imagens por SRD: linear e logarítmica. Na primeira, obtêm-se uma imagem com tom de cinza intermediário (Figura 1), enquanto que no segundo tipo, o contraste radiográfico e a quantidade de ruído são aumentados (Figura 2). Versteeg & Van der Stelt, em 1995, compararam estes dois tipos de subtração na avaliação de lesões criadas artificialmente por meio do computador. A análise foi feita por 20 avaliadores que decidiram em uma escala de 5 pontos, se existia ou não a presença de uma lesão. Os autores concluíram que a subtração radiográfica digital logarítmica proporcionou informações de diagnóstico melhores que a subtração linear, mas afirmaram que estudos clínicos ainda eram necessários para excluir as limitações de lesões criadas artificialmente. Na nova versão 5.0.12 do programa EMAGO®, foi criado uma nova ferramenta denominada "advanced subtraction" onde existe uma combinação dos comandos "reconstruction", "gamma correction" e "linear subtraction", o que significa que o programa automaticamente subtrai as duas imagens, rotaciona uma imagem em relação à outra, calibra os níveis de cinza e cria a imagem de subtração radiográfica digital linear em tempo real.

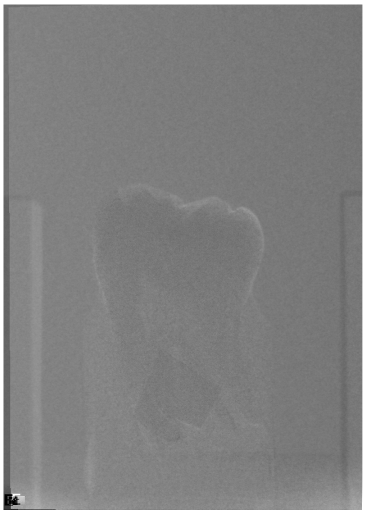

Figura 1 - Exemplo de SRD linear

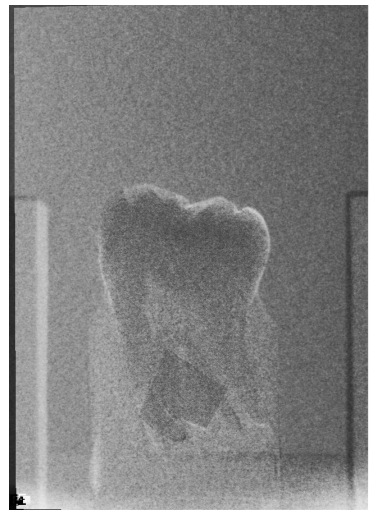

Figura 2 - Exemplo de SRD logarítmica

A lesão de cárie e a remineralização não é uma radioluscência bem definida com o aumento ou diminuição do nível de calcificação para a periferia da lesão. Portanto, mensurações da extensão da lesão e monitoramento da remineralização são difíceis de serem feitas com altos valores de acurácia (Eberhard *et al.*, 2000). A progressão de descalcificações *in vitro* tem sido avaliada e quantificada por meio de técnicas como a micro-radiografia, microscopia de luz polarizada, teste de dureza do esmalte seccionado longitudinalmente, dosagens bioquímicas de Ca e P_i e SRD. Entretanto, existe a necessidade de métodos que monitorem o estado mineral e também correlacionem entre si, para que uma comparação dos resultados de estudos *in vitro*, *in situ* e *in vivo* possam ser realizados. O método ideal deve ser capaz de permitir medidas sequencionais e ser quantitativo para o ganho e perda mineral. Ganss *et al.* (2005) correlacionaram valores de mensurações de perda mineral erosiva por meio de quatro diferentes métodos: dosagens de Ca e P_i, profilometria de superfície e microradiografia longitudinal. Como resultado, obteve uma correlação positiva entre estes métodos.

Em imagens radiográficas digitais, para o monitoramento de lesões de cárie, existem possibilidades de avaliação subjetiva por meio de interpretação e comparação direta das duas imagens visualizadas; e, uma avaliação objetiva por meio da densidade radiográfica medida por meio do histograma. Entretanto, ao se propor um novo método de aquisição de imagem, como os equipamentos de radiografias digitais e recursos tecnológicos como a SRD, faz-se necessário que os mesmos sejam avaliados *in vitro* e correlacionados com técnicas já estabelecidas para que a comparação de estudos *in vitro*, *in situ* e *in vivo* possam ser comparados e uma técnica de diagnóstico com uma acurácia satisfatória seja estabelecida para o seu real uso *in vivo*.

Diante do exposto esta pesquisa objetiva avaliar:

A - Comparar duas metodologias de remineralização *in vitro* do esmalte dentário por meio de solução remineralizante e exposição ao flúor, usando como métodos de avaliação: dosagem bioquímica, teste de dureza do esmalte, microscopia de luz polarizada e imagens de subtração radiográfica digital.

B - Comparar as dosagens bioquímicas de cálcio e fósforo, avaliação da perda mineral por meio do teste de dureza do esmalte e análises de densidade por meio do histograma em imagens de microscopia de luz polarizada e subtração radiográfica digital na avaliação quantitativa de mudanças minerais em um modelo *in vitro.*

C - Avaliar a viabilidade do uso de dois diferentes equipamentos de radiografia digital (DIGORA OpTime® / placa PSP e CDR Wireless® / sensor CMOS) no diagnóstico de desmineralizações do esmalte; e, avaliar a acurácia de radiografias digitais convencionais e três métodos de subtração radiográfica digital (linear, avançada e logarítmica) na detecção de mudanças minerais em esmalte *in vitro*.

D - Comparar o ruído e a reprodutibilidade de imagens de subtração radiográfica digital linear e logarítmica produzidas a partir de imagens feitas com dois diferentes sistemas de imagens digitais em três regiões de interesse, com variação do tamanho e estrutura.

CAPÍTULO 1

(Artigo enviado para "Caries Research" / Anexo 2)

TITLE PAGE

Title of the paper:

In vitro remineralization of artificial carious lesions assessed by subtraction images

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Short title:

Dental enamel remineralization

Key Words: Fluoride, Artificial saliva, Subtraction radiography, Remineralization Dental caries, *In vitro* model

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Abstract

The aim of this *in vitro* study was to compare two remineralization protocols of artificial carious lesions in enamel, for 4 and 8 weeks, subjected to continuous immersion in artificial saliva, and additional fluoride treatment. Determination of calcium (Ca) and phosphorus (P_i) in the saliva solution, subtraction images, cross-sectional hardness test and polarized light microscopy were used as evaluation methods. The concentrations of Ca and P_i in the saliva after the treatments were significantly lower ($p<0.05$) than the original solution and digital subtraction showed differences between the images. However, the hardness test and polarized light microscopy did not detect any changes.

Introduction

Caries initiation is associated with demineralization of subsurface tooth enamel. Calcium and phosphate are lost from the surface enamel, resulting in the formation of a subsurface lesion. At this early stage, the caries lesion is reversible via a remineralization process involving the diffusion of calcium (Ca) and phosphorus (P_i) ions into the subsurface lesion to restore lost structure. This remineralization phenomenon, using calcium phosphate solutions only or with additional fluoride (F) treatment, has been shown to exist [Ingram & Edgar, 1994]. Fluoride, in its several forms, has been an outstanding means of preventing caries. Indeed, remineralization is possible even at a high degree of initial mineral loss, where it might have been considered that the caries process had passed a "point of no return" [ten Cate, 2001; Mukai & ten Cate, 2002].

A wide range of techniques have been used to measure the changes occurring in dental tissue, including tests to quantify changes in physical properties such as hardness test, changes in chemical composition, or polarized light microscopy [Argenta *et al*., 2003; Ganss *et al*., 2005]. In addition, when two radiographs are recorded with controlled projection angles and then subtracted, theoretically all unchanged anatomical background structures are cancelled, and these areas are displayed in a neutral grey shade in the subtraction image; regions that have changed between the radiographic examinations are displayed in darker (loss) or lighter (gain) shades of gray. For the detection of caries and monitoring of remineralization therapy, digital subtraction images have made possible the careful analysis of time lapse radiographs [Wenzel & Halse, 1992; Halse *et al*., 1994].

The aim of the current study was to compare an *in vitro* methodology using two protocols of dental enamel remineralization by exposure to calcifying and F fluids. The evaluation methods used were: chemical analysis, cross-sectional hardness test, polarized light microscopy and digital subtraction images.

Materials and Methods

Tooth preparation and lesion formation

One hundred previously extracted impacted third molars were sterilized by storage in 10% buffered formalin solution (pH 7.0) for 7 days [Dominici *et al*., 2001]. They were rinsed off with deionized water and mounted in an individual acrylic resin base, except the crowns, giving the teeth a good stability for the radiographs. A piece of aluminum was also inserted in the acrylic base for use as reference points during alignment of the radiographs. The crowns were then coated with nail varnish, except for an exposed window on one of the proximal surfaces of about 7 mm^2. Artificial caries-like lesions were induced by immersion of each tooth in 14 ml of a demineralizing solution, containing 0.05 M acetate buffer, 1.3 mM Ca, 0.77 mM P_i and 0.03 ppm F (pH 4.8) at 37 °C, for 75 days [Haiter-Neto *et al*., 2005]. After 60 days of incubation, the demineralizing solution was changed in order to avoid Ca and P_i saturation.

Remineralization of artificial caries-like lesions

After the demineralizing process, 20 teeth were kept as controls. The remaining 80 teeth were randomly assigned into four groups (*n*=20), subjected to two different experimental protocols for 4 and 8 weeks. Protocol A involved continuous remineralization in an artificial saliva solution, and protocol B was as for protocol A with additional treatment with F. The artificial saliva solution contained 1.5 mM Ca, 0.9 mM P_i, 150 mM KCl in a buffer solution of 20 mM tris (hydroxymethyl)-aminomethane at pH 7.0 [Featherstone *et al*., 1986; Serra & Cury, 1992]. The specimens were individually placed in 7 ml of artificial saliva solution, which was changed every week, and kept at 37 °C. Three times a day the specimens from protocol B were taken out of the saliva solution, rinsed with deionized water and placed, individually, in 7 ml of a F solution for 5 min at 150 rpm of agitation (TE-140®,Tecnal Equipment, São Paulo, Brazil). The F solution contained 280 ppm F (NaF) to simulate the dilution that occurs in the oral cavity when fluoridated toothpaste (1100 µg F/g) is used [Duke & Forward, 1982]. The specimens were then taken out of the F solution, rinsed with deionized water and replaced in the original artificial saliva solution.

Analysis

The artificial saliva solution, which was replaced every week, was chemically analyzed to determine the concentrations of Ca and P_i in the fresh solution and during the experimental protocols. Phosphorus concentration was determined using a colorimetric method [Fiske & Subbarow, 1925]. The concentration of Ca was determined by atomic absorption spectrophotometry, using lanthanum to suppress phosphate interference [Cury *et al.*, 2003].

Radiographs were taken prior to and after the remineralization period. GE 1000® X-ray equipment (General Electric Co., Milwaukee, WI, USA) was used operating at 65 kVp, 10 mA and 0.25 s with DIGORA OpTime® (Orion Corp./Soredex, Helsinque, Finland) photostimulable storage phosphor plates. An acrylic device was also used to standardize the relationship among the teeth, the x-ray beam indicator device and the image receptors in a reproducible way. In addition, 2.5 cm thick acrylic was positioned in front of the tooth to simulate the soft tissues [Haiter-Neto *et al.*, 2005]. The images were manipulated using EMAGO®/advanced 5.0.12 software (Oral Diagnosis Systems, Amsterdan, The Netherlands) and digital subtraction images were obtained.

The enamel areas were submitted to cross-sectional hardness analysis. The crowns were separated from the roots and cut in half vertically through the centre of the test areas. Half of each crown was embedded in methylmethacrylate resin so that the cut section of the test area and the underlying normal enamel were exposed. This surface was then serially polished. The hardness profile of each lesion was measured across three positions located at a quarter, half and three-quarters of the width of the lesion, starting at 10 µm from the enamel surface. Indentations were made with the long axis of the diamond parallel to the outer enamel surface, in a total of 18 indentations across the lesion and into the underlying sound enamel, with 25 g load for 5 s. The values of Knoop hardness number (KHN) were converted to mineral content (volume % mineral) using the relation: mineral content=4.3($\sqrt{}$KHN)+11.3 [Featherstone *et al.*, 1983]. The data set representing each artificial carious lesion (in each enamel block) was fitted to a curve. The area under the lesion tracing was calculated by means of the trapezoidal rule (in units of volume % mineral x micrometers), and subtracted from the normal enamel value to give the mineral loss area (parameter ΔZ) [Featherstone & Zero, 1992].

After the cross-sectional hardness analysis, the embedded enamel blocks were sectioned in order to obtain longitudinal slices of 100 µm (±10 µm). These sections were mounted for examination under a polarizing light microscope at 5x magnification (DMLSP, Leica, Wetzlar, Germany). Digital images were obtained with specific software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA).

The digital images from the polarizing light microscopy and the digital subtraction images were visually inspected by one of the researchers (J.A.B.C.), by direct comparison of the treatment groups with the control demineralization group and the presence of an increased density, respectively. SAS statistical software was used to conduct the statistical analysis of the inorganic concentration in the saliva and hardness data. Independent group two-way ANOVA and Dunnet's test were performed to ascertain if there were differences between the two treatment groups, and differences between the treatment groups and the demineralization control group, respectively.

Results

The inorganic concentration of the artificial saliva solution, during and after the remineralization period, demonstrated that the enamel incorporated Ca and P_i. A mean reduction of 19% and 21% in Ca concentration per week in the artificial saliva solution was observed in protocol A at 4 and 8 weeks, respectively; and for protocol B the reduction was 43% and 40%. The reduction in P_i concentration was 28% and 26% in protocol B (table 1). Dunnet's test showed that the concentration of Ca and P_i in the artificial saliva solution after treatments differed significantly from the original solution in all groups, except P_i in protocol A. Using ANOVA, a comparison between the periods (4 and 8 weeks) and the protocols (A and B) was also conducted, and the differences between them were significant ($p<0.05$). In protocol B, independent of the time used, lower values of Ca concentration were demonstrated compared to protocol A, suggesting higher incorporation by the enamel; the same pattern was observed for P_i.

Differences in density could be observed by direct visual comparison of the digital radiographs taken before and after both remineralization protocols and periods of time, confirmed by an increased density (lighter area) shown on the subtraction images (figure 1). The magnitude of the recorded alterations varied from barely visible to quite pronounced in a mean of 7 out of the 20 teeth in all groups.

In the mineral loss analysis, there were no statistically significant differences between the groups. In addition, the difference between the remineralization groups and the demineralization control group was not statistically significant. From the absolute values, it can be observed that there is a slightly higher mineral loss area (ΔZ) in the demineralization group. Similarly, the digital images from polarizing light microscopy did not demonstrate visual differences between the remineralization groups and the demineralization control group (figure 2).

Discussion

The demineralization protocol used in the present study has been successfully evaluated before by Haiter-Neto *et al*. [2005], who showed that mineral loss can be detected by digital subtraction. Third molars were immersed in a demineralizing solution for 60, 75, 90 and 120 days in order to induce subsurface demineralization. In this study, the period of 75 days was chosen, based on a pilot study; after this period, demineralization was radiographically visible although not as deep as after longer periods.

The remineralization process evaluated in this study was validated by the decrease in the concentrations of Ca and P_i in the artificial saliva solution after treatments [Al-Khateeb *et al.*, 1997]. Fluoride ions were able to enhance the percentage of mineral content gained per week, i.e. in protocol B a better recovery of the mineral content of the pre-formed lesions was obtained [ten Cate *et al.*, 1985; Yamazaki *et al.*, 2007]. Huysmans & Longbotton [2004] reported that if one considers the definition of caries progress as de/remineralization imbalance leading to net mineral loss, it seems most logical to take mineral content as the preferred parameter to follow this imbalance.

During recent years, digital subtraction images have been established as a sensitive technique for the detection of small changes in density [Maggio *et al.*, 1990]. In this study, digital radiographs of the same specimens before and after the treatment were subtracted and the differences were visualized as lighter shades of grey, suggesting a mineral gain. However, it could be observed on conventional digital images that the carious-like lesions did not recovery completely; the presence of radiolucency was still noted in all treatment groups.

Hardness measurements have been considered to be a valuable method that reflects physical changes of acid-softened surfaces [Fujimaru *et al.*, 2003]. However, Ganss *et al.* [2005] stated that with increasing exposure to acids, hardness decreases to a minimum, whereas the dissolution of mineral increases further; so this method is limited to the initial stages of erosion. Based on this concept, it seems that in this study, the mineral loss area (ΔZ) of the treatment groups could not reach significance compared to the control demineralization group, because of the relatively deeper lesions necessary for this study. Thus, a complete or almost complete re-hardening of the lesion did not occur. In addition, the images from polarized light microscopy did not demonstrate any difference between the experimental groups and the demineralizing control group. Hardness and polarized light measurements of the mineral contents of the same lesion area before and after the treatment regimen were not possible as these are destructive analyses. Nevertheless, a control group of a relatively favorable sample size required for comparison was used in this study, and it must be pointed out that each tooth may behave uniquely, even though selection criteria were applied.

Greater remineralization could possibly be achieved by using a longer term remineralization protocol [ten Cate, 2001] and/or reducing solution pH [Alves *et al*., 2007]. In the present study, the 8-week remineralization period seemed to be too short for a significant mineral gain, ten Cate [2001] presented a simple mechanistic model of remineralization, where either diffusion or precipitation is considered as the ‘rate-limiting’ step. Care has to be taken to provide a protocol of ‘slow’ precipitation, leading to a constant concentration of calcium and phosphate within the pores. The rate of mineral deposition also depends on local factors like pH, the presence of seed crystals or matrix, and the surface area available for crystal growth. A remineralization solution with low pH may be a good option to test in a future study.

This *in vitro* experiment concluded that although the dosages of Ca and P_i in the artificial saliva showed differences between the groups, suggesting mineral gain, this was not detected in the evaluation of cross-sectional hardness and polarized light microscopy. Mineral gain could also be observed in the digital subtraction images, providing a method to longitudinally monitor the remineralization of incipient proximal carious lesions; the accuracy of this method should be the next step to be tested.

Acknowledgements

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Legends

Figure 1.

A subtraction image of a tooth presented to protocol B, after 4 weeks of remineralization. In the zoom image (indicated by the arrow), increased density (lighter area) on the lesion area and also lighter areas of structural noise can be seen.

Figure 2.

Polarized light microscope digital images of one tooth on each group: A, control group; B, protocol A after 4 weeks; C, protocol A after 8 weeks; D, protocol B after 4 weeks; E, protocol B after 8 weeks.

Figures

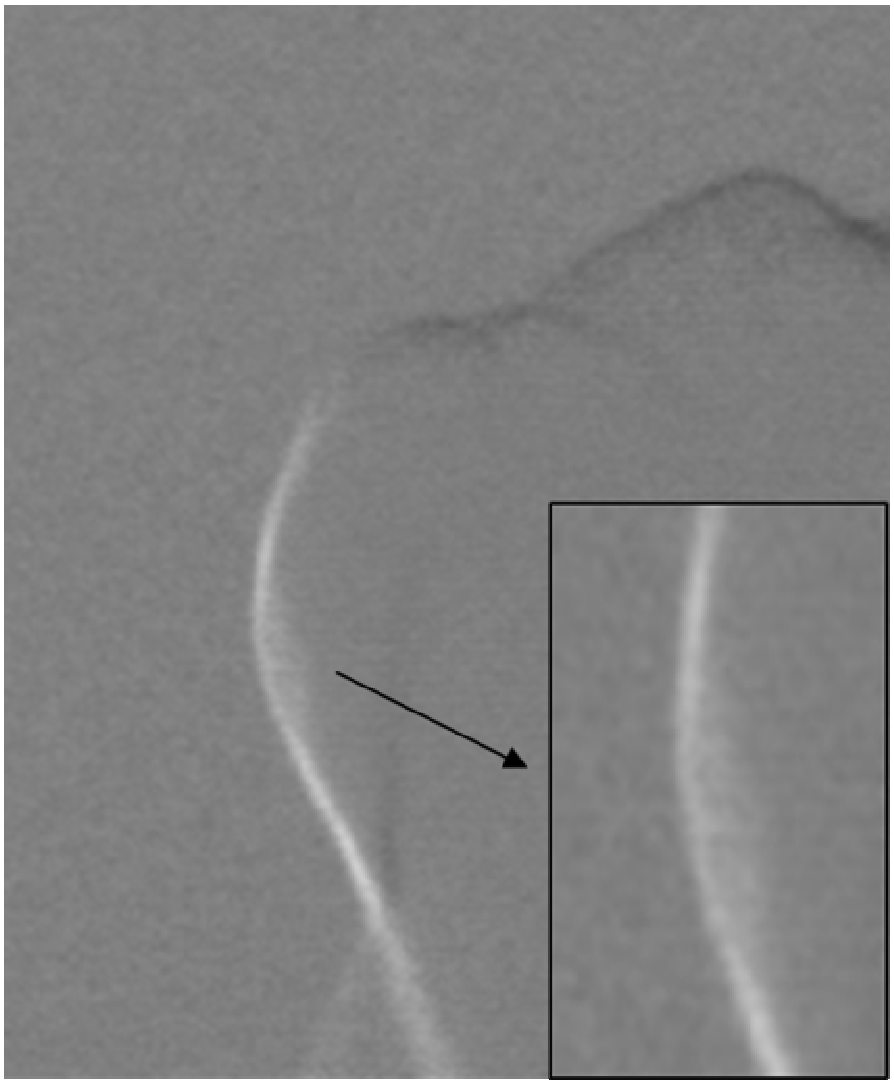

Figure 1.

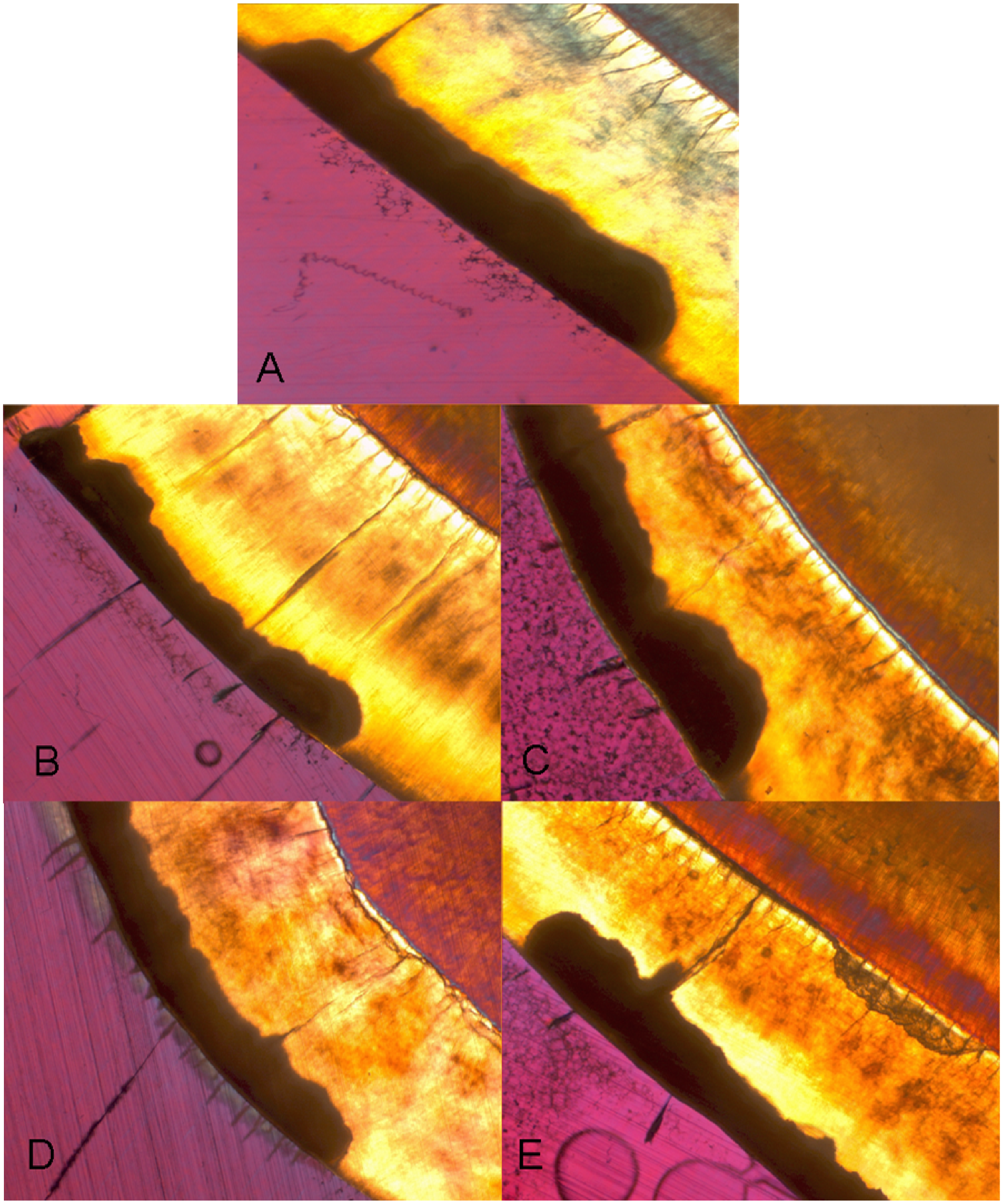

Figure 2

Table

Table 1. **Calcium and inorganic phosphorus concentration (mM) in artificial saliva solution and mineral loss area (ΔZ; mean ± Standard deviation (SD)) of protocol A (continuous remineralization) and protocol B (continuous remineralization + fluoride treatment) after 4 and 8 weeks (n=20).**

Variables	**Time (weeks)**	**Protocols**	
		A	**B**
Calcium	**4**	1.38 ± 0.04 * Aa	0.98 ± 0.09* Bb
	8	1.34 ± 0.05 * Ab	1.02 ± 0.04* Ba
Phosphorus	**4**	0.85 ± 0.02 Ab	0.62 ± 0.05* Bb
	8	0.86 ± 0.02 Aa	0.64 ± 0.03* Ba
Mineral loss	**4**	5907.2 ± 1054.9 Aa	6132.2 ± 1147.8 Aa
	8	6082.9 ± 1303.5 Aa	5883.2 ± 1087.8 Aa

Mean (mM) ± SD of calcium and phosphorus concentration of the original artificial saliva solution = 1.69 ± 0.05 and 0.86 ± 0.01, respectively.
Mean (ΔZ) ± SD of mineral loss area of the demineralization group (n=20) = 6299.9 ± 1463.9
* Statistically different from the concentration in the original solution by Dunnet's test (p<0.05).
Means with the same letter are not significantly different by ANOVA test, comparing capital letters in the row and small letters in the column (p < 0.05)

CAPÍTULO 2

TITLE PAGE

Title of the paper:

Comparative study of different techniques to quantify dental enamel remineralization

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Short title:

Quantification of dental enamel remineralization

Key Words: Digital radiography, Subtraction images, Remineralization

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Abstract

The objective of this study was to compare Calcium (Ca)/Phosphorus (P_i) analysis, mineral loss area calculated from cross-section hardness test, and density measurements in polarized light microscope (PLM) and digital subtraction images to quantify *in vitro* protocols of dental enamel remineralization. Third molars samples were subjected to two different remineralization protocols for 4 and 8 weeks. Remineralization of each sample was estimated by the five methods. Ca/P_i analysis and density measurements in PLM images revealed statistically significant difference between the protocols. Ca and P_i analysis was also highly associated in all treatment groups, indicating that it was the most sensitive method to quantitatively monitor mineral changes on dental enamel.

Introduction

A carious lesion and remineralization is not a well-defined radiolucency as the degree of calcification decrease/increases towards the periphery of the lesion. Thus, measurements of the extent of a carious lesion and monitoring of remineralization are difficult to perform accurately [Eberhard *et al*., 2000]. Progression of *in vitro* decalcification has been evaluated and quantified by microradiography, polarized light microscopy, light microscopy using stain reagents, hardness measurements, calcium / phosphorus analysis and subtraction radiography. The use of quantitative image comparison and image analysis promises to give even more information on this process [Klinger & Wiedmann, 1985].

There is a need for methods that can monitor remineralization and mineral status and also be correlated, to have better comparison of *in vitro*, *in situ* and *in vivo* studies results, using similar experimental settings. A method meeting all requirements should be suitable to allow sequentional measurements and be quantitative for mineral loss as well as mineral gain. Ganss *et al*. [2005] related erosive mineral loss values as measured by four different methods: calcium analysis, phosphorus analysis, surface profilometry and longitudinal microradiography, a good linear correlation was found between these methods.

The objective of this investigation was to compare calcium (Ca) / phosphorus (P_i) analysis, mineral loss area calculated from cross-section hardness test, and density measurements in polarized light microscope and digital subtraction images on the quantitative assessment of mineral changes in an *in vitro* model, which closely simulate natural enamel remineralization.

Materials and Methods

From an *in vitro* remineralization study, eighty samples were used. The protocol used has been published earlier and is summarized here. Previously extracted third molars were selected, mounted in an individual acrylic resin base; the crowns were coated with nail varnish, except for an exposed window on one of the proximal surfaces of about 7 mm^2. Then, they were individually immersed in a demineralizing solution for 75 days [Haiter-Neto *et al.*, 2005]. After the demineralizing process, the 80 samples were randomly assigned in four groups (n=20), subjected for 4 and 8 weeks to two different experimental protocols, being either continuous remineralization in artificial saliva solution or remineralization with additional treatment with Fluoride (F).

Chemical analysis was determined in the artificial saliva solution during the remineralization process. Calcium (Ca) concentration was determined by atomic absorption spectrophotometry, which was performed in the presence of lanthanum to suppress phosphate interference. The solution was also analyzed for phosphorus (P_i) concentration by a colorimetric method [Fiske & Subbarow, 1925].

Digital radiographs were taken prior to and after remineralization period with DIGORA OpTime® (Orion Corp./Soredex, Helsinque, Filand) photostimulated storage phosphor (PSP) plates. An acrylic device was used to standardize the relationship among teeth, x-ray bean indicator device and image receptor in a reproducible way. At this time, the images were not manipulated and they were stored as tagged image file format (TIFF). By EMAGO® / advanced 5.0.12 software (Oral Diagnosis Systems, Amsterdan, the Netherlands), digital subtraction image (DSI) were obtained for each tooth. The method of subtraction used was the advanced subtraction; that allowed a combination of reconstruction, gamma correction and linear subtraction, i.e. the interactive software which transposed the two images, translated one image with respect to the other, automatically calibrates the gray levels, and then performed a subtraction in real time.

Afterwards, the enamel area was submitted to cross-sectional hardness (CH) analysis. The crowns were separated from the roots and cut in half vertically through the centre of the test areas. The halves of each crown were embedded in methylmethacrylate resin so that the cut section of the test area and the underlying normal enamel were exposed. This surface was then serially polished. The hardness profile on each lesion was measured across three positions located at ¼,

½ and ¾ of the width of the lesion, starting at 10 µm from the enamel surface. Indentations were made with the long axis of the diamond parallel to the outer enamel surface, in a total of 18 indentations across the lesion and into the underlying sound enamel, with 25 g load for 5 s. The values of knoop hardness number (KHN) were converted to mineral content (volume % mineral) using the relation: mineral content = 4.3 ($\sqrt{KHN}$) + 11.3 [Featherstone *et al.*, 1983]. The data set representing each artificial carious lesion (in each enamel block) was fitted to a curve. The area under the lesion tracing was calculated by means of the trapezoidal rule (in units of volume percent mineral x µm), and subtracted from the normal enamel value to give the mineral loss area (parameter ΔZ) [Featherstone & Zero, 1992].

After the cross-sectional hardness analysis, the embebbed enamel blocks were sectioned in order to obtain longitudinal slices of 100 µm (± 10). These sections were mounted for examination under a polarizing light microscope (PLM) at 10 x magnification (DMLSP, Leica, Wetzlar, Germany). Digital images were captured with specific software (Image-Pro Plus, Media Cybernetics, Silver Spring USA), stored as TIFF, black and white 8 bit format.

The mean density value of selected regions of interest (ROI) in DSI and PLM images were also obtained by EMAGO® / advanced 5.0.12 software (Oral Diagnosis Systems, Amsterdan, the Netherlands). The histogram command was used to assess the mean density value of three ROI enclosing the proximal lesion area and three ROI in sound enamel. The average of the three ROI in the lesion and in the sound enamel was subtracted and the difference was used as a parameter for the density change occurring in the DSI and in the PLM image.

Correlation coefficients of Pearson were calculated for the concentration of Ca and P_i, the mineral loss area (ΔZ) from the cross-section hardeness test, and the mean density value differences of the histogram from DSI and PLM images. Also, Independent group two-way ANOVA was performed to ascertain if there were differences between the remineralization protocols in all five evaluated methods.

Results

Table 1 shows the r coefficient of Pearson value off all correlated methods, indicating the strengths of associations between them in each protocol and period of time. The correlation between Ca and P_i analysis were high and extremely significant in all groups. The mean density value difference of the histogram in the PLM image was also significantly correlated to Ca and P_i analysis, and to cross-section hardness test, only in protocol B / 8 weeks and protocol A / 4 weeks, respectively.

By ANOVA a comparison between the periods (4 and 8 weeks) and the protocols (continuous remineralization in artificial saliva solution or remineralization with additional treatment with F) did not reveal statistically significant difference in all evaluated methods, except for the Ca and P_i analysis (that has been given in a different publication) and the mean density difference of the histogram in PLM images (Table 2).

Discussion

The protocols used in this study produced a dental enamel remineralization mimicking the radiographic appearance of proximal lesions quite well. However the progression of this treatment could not be well monitored, quantitatively, by the mean density value of the histogram in DSI; the method failed to reveal differences between the two remineralization protocols. These results are consistent with the findings of Eberhard *et al*. [2000] who studied a density method of reducing demineralization in 14 extracted human teeth, and the subtraction images failed to reveal statistically significant grayscale changes between a control method and a method of remineralization.

The measurement of the mean pixel value by the histogram as a parameter of quantitatively measuring the mean density value of selected ROI has been widely used and is an important aspect of digital imaging, providing a means to make precise and reliable diagnoses that were not possible in the past. This method can be applied to conventional radiography and subtraction images [Bittar-Cortez *et al.*, 2006]. The calculation of the mean density value was limited, in this study, to subtraction images; the reason for that is the small size of the proximal lesions that in conventional radiography cannot be well determined.

In this study, this method of calculating the mean density value in selected ROI was also tested in PLM images. Although the method did not correlate equally in all remineralization protocols, interpretation of the results must therefore take into consideration that the absolute values differences in the mean density value in the PLM images (table 2) demonstrated statistically significant lower values when F was used, i.e. the mean density value of the lesion where closer to the values of the sound enamel. Thereby, the introduced method of measuring mean density value by the histogram in PLM images might facilitate the detection of longitudinal monitoring of artificial caries-like lesions.

Cross-section hardness analysis could not detect differences between the remineralization protocols, i.e. that the indentations measurements could not reflect the difference of mineral gained in the four treatment groups. This could be expected to a certain extent, since there are statements that this method is limited to only initial stages of erosion [Ganss *et al.*, 2005], on the other hand, Haiter-Neto et. al. [2005] have used the CH test as a validation method of the true absence or presence of enamel subsurface demineralization of lesion varying from 230 to 410 µm, and the method was able to detect differences between the periods of demineralization.

Results from Ca and P_i analysis showed a strong correlation, which is similar to the findings of a study assessing methods of erosive mineral loss [Ganss *et al.*, 2005]. The chemical analysis and density values in PLM images were also the only methods capable of distinguishing between the two remineralization protocols. Different result was found by Eraso *et al.* [2007], where a linear relationship between subtraction units and calcium loss was obtained.

Within the limitation of this *in vitro* study, it was concluded that the chemical analysis is the best method to determine differences between remineralization protocols, however the mean pixel value measured by the histogram in ROI, appears to be an alternative for monitoring remineralization protocols.

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Tables

Table 1. Coefficient of Pearson r (p-value) off all correlated techniques.

Protocol / Period	Techniques	P_i	CH	PLM	DSI
A / 4 weeks	**Calcium analalysis (Ca)**	**0.603 (p=0.005)**	0.217 (p=0.354)	0,366 (p=0.112)	0.021 (p=0.929)
	Phosphorus analysis (P_i)	**1**	-0.269 (p=0.251)	-0,013 (p=0.957)	-0.241 (p=0.305)
	Cross-section hardness (CH)		**1**	**0.730 (p=0.000)**	0.374 (p=0.105)
	Polarized light microscope (PLM)			**1**	0,176 (p=0.458)
A / 8 weeks	**Calcium analalysis (Ca)**	**0.675 (p=0.002)**	-0.122 (p=0.619)	0.190 (p=0.435)	-0.128 (p=0.601)
	Phosphorus analysis (P_i)	**1**	-0.028 (p=0.910)	0.086 (p=0.726)	-0.281 (p=0.244)
	Cross-section hardness (CH)		**1**	0.039 (p=0.878)	0.055 (p=0.822)
	Polarized light microscope (PLM)			**1**	-0.306 (p=0.203)
B / 4 weeks	**Calcium analalysis (Ca)**	**0.924 (p=0.000)**	-0.247 (p=0.295)	0.102 (p=0.668)	-0.112 (p=0.628)
	Phosphorus analysis (P_i)	**1**	-0.209 (p=0.375)	0.008 (p=0.972)	0.022 (p=0.927)
	Cross-section hardness (CH)		**1**	-0.115 (p=0.630)	0.090 (p=0.705)
	Polarized light microscope (PLM)			**1**	-0.413 (p=0.070)
B / 8 weeks	**Calcium analalysis (Ca)**	**0.918 (p=0.000)**	0.048 (p=0.841)	**-0.607 (p=0.005)**	0.125 (p=0.600)
	Phosphorus analysis (P_i)	**1**	-0.194 (p=0.411)	**-0.635 (p=0.003)**	0.017 (p=0.944)
	Cross-section hardness (CH)		**1**	-0.061 (p=0.795)	0.338 (p=0.145)
	Polarized light microscope (PLM)			**1**	-0.337 (p=0.146)

Level of significance was set at 5 %

Table 2. Differences in the mean density value of the histogram ± standard deviation in the DSI and PLM images of protocol A (continuous remineralization) and protocol B (continuous remineralization + fluoride treatment) after 4 and 8 weeks (n=20).

Methods	Period (weeks)	Protocols	
		A	B
Density in DSI	4	15.14 ± 6.79 Aa	18.54 ± 6.70 Aa
	8	17.78 ± 7.53 Aa	16.87 ± 6.53 Aa
Density in PLM images	4	136.63 ± 23.14 Aa	122.79 ± 21.29 Ba
	8	141.85 ± 25.26 Aa	126.70 ± 26.10 Ba

Means with the same letter are not significantly different by ANOVA test, comparing capital letters in the row and small letters in the column ($p < 0.05$)

CAPÍTULO 3

TITLE PAGE

Title of the paper:

***In vitro* comparison of digital and subtraction images for approximal artificial caries-like lesions and mineral changes diagnostic accuracy**

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Short title:

Digital and subtraction images in mineral changes diagnosis

Key Words: Digital radiography, Subtraction images, Remineralization, Image processing, Diagnosis

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Abstract

Considerable research during the past two decades has focused upon the development of new technologies for the detection and monitoring of dental caries. The purpose of the present study were twofold: to assess the feasibility of using two different systems of digital radiography, photostimulable storage phosphor (PSP) plate and complementary metal oxide semiconductor (CMOS) sensor, for the diagnosis of approximal enamel demineralization; and the accuracy of digital conventional radiographs (DCR) and three methods of digital subtraction images (DSI): linear, advanced and logarithmic, to detect mineral changes on human enamel *in vitro.* Artificial caries-like lesions on 100 approximal surfaces of extracted third molars were produced. Eighty teeth were submitted to two different remineralization protocols in two periods of time. Digital radiographs were taken before and after the remineralization protocols. Five examiners assessed demineralization and mineral changes caused by two remineralization protocols on DCR placed side by side and three methods of DSI. Intra and inter-examiner reliability Kappa statistics were calculated. Calcium/phosphorus analysis and the placement of the teeth on the remineralization solution was the true state of mineral gained. CMOS sensor was significantly more accurate than PSP plate, using DCR on demineralization diagnosis, and using DCR and DSI on mineral changes diagnosis. It was concluded that (a) CMOS sensor was more accurate than PSP plate on the diagnosis of artificial caries-like lesions and mineral changes monitoring; and (b) linear DSI can be a valuable method to disclose an intensity increase, as a sign of mineral gained.

Introduction

With the advent of remineralization therapies and the new, conservative approach to restoration placement, interest in detecting and monitoring subclinical, precavitaded lesions has increased. Nowadays, the caries preventive effect of fluoride is without any doubt [Ingram and Edgar, 1994]. Fluoride has been shown to actively affect the formation of lesions as well as their remineralization or arrestment [Takagi *et al*., 2000; Lagerweij and ten Cate, 2002]. The increased understanding of clinicians about the process of primary and secondary prevention and detection of lesions, to which these therapies may be applied, is one of the current goals in caries management.

Cariologists and clinicians are currently interested in the detection of early carious lesions of the kind that can be reversed following fluoride or similar interventions. Thereby, non-destructive methods for quantitatively investigating progression (demineralization) or recovery (remineralization) of carious lesions in enamel could be very useful to monitor the changes related to preventive measures over time. Several techniques such as electrical resistance [Wang *et al*., 2005], quantitative light-induced fluorescence [Pretty *et al*., 2003] and laser fluorescence [Lussi *et al*., 2001] have been developed in recent years to measure mineral changes on human enamel. However, there is still no generally accepted method of measuring mineral changes over remineralization protocols [Wang *et al*., 2005], especially in approximal surface lesions.

Digital radiographic systems, compared to conventional radiography, shows possibilities for equally diagnostic accuracy of caries lesions [Wenzel 2006]. These systems also make easier the use of another digital imaging method, such as digital subtraction images (DSI), that has been established as a sensitive technique for the detection of small changes in hard tissues, and can also represent an effective tool for the cariology community [Wenzel *et al*., 2000]. In the diagnosis of proximal or occlusal demineralization, DSI was found to be more accurate with higher values of sensibility [Haiter-Neto *et al*., 2005; Ricketts *et al*., 2007]. An increase of radiographic density in areas of occlusal and approximal demineralization after the use of fluoride solution has been also disclosed by DSI [Halse *et al*., 1990; Wenzel & Halse, 1992], but there was not a comparison of different methods of subtraction images on mineral changes diagnosis.

The purpose of the present study were twofold: to assess the feasibility of using two different equipments of digital radiography, DIGORA OpTime® (Orion Corp./Soredex, Helsinque, Finlândia) PSP plate; and CDR Wireless® (Schick Technologies, NY, USA), CMOS sensor, for diagnosis of approximal enamel demineralization; and the accuracy of DCR and three methods of DSI (linear, advanced and logarithmic) to detect mineral changes on human enamel *in vitro*. The null hypothesis is that DCR is less accurate than DSI in mineral changes detection of approximal artificial caries-like lesions. The technical description of the remineralization methodology is outside the scope of this article.

Materials and Methods

Preparation of tooth

A detailed account of the materials and methods of sample preparation has been given in a different publication. Therefore, only a relevant summary is provided here.

Caries-like lesions were formed on one of the proximal surfaces in the enamel of 100 extracted human molars by means of a pH 4.8 demineralizing solution, for 75 days [Haiter-Neto *et al*., 2005]. Twenty teeth were kept as control (demineralization group) and the remaining 80 teeth were randomly divided into four groups (n=20) subjected for 4 and 8 weeks to two different experimental protocols, being either continuous remineralization in an artificial saliva solution (procedure A) or remineralization as for A with additional immersion in a fluoride solution (procedure B). Chemical analysis of calcium (Ca) and phosphorus (P_i) in the artificial saliva solution after the treatments demonstrated a mineral reduction, indicating that all 80 teeth have gained mineral. The protocol B obtained a higher mineral reduction in the artificial saliva than in protocol A.

Before the demineralization, the teeth were mounted in individual acrylic resin base, except the crowns, giving the teeth a proper stability to take the radiographs. A peace of aluminum was also inserted in the acrylic base to be used as reference points during alignment of longitudinal radiographs.

Radiographic Technique

Standardized radiographs were taken prior to and after remineralization period. Two systems of digital radiographs were used: DIGORA OpTime® (Orion Corp./Soredex, Helsinque, Filand) photostimulable storage phosphor (PSP) plate; and CDR Wireless® (Schick Technologies, NY, USA), complemetary metal oxide semiconductor (CMOS) sensor. A GE 1000® X-ray equipment (General Electric Co., Milwaukee, WI, USA) was used operating at 65 kVp, 10 mA and 0.1 s for the CMOS sensor and 0.25 s for the PSP plates. An acrylic device was used to standardize the relationship between teeth, x-ray beam indicator device and image receptors in a reproducible way. Additionally, 2.5 cm thick acrylic was positioned in front of the tooth to simulate the soft tissues.

Digital subtraction images (DSI)

Before the subtraction procedure, the images were processed using ACDsee® 6.0 software (ACD systems Ltd, British Columbia, Canada) so as to duplicate the images of the control demineralizing group and to resize the images obtained from PSP plates. The original matriz size of the CMOS and PSP system was 640 x 900 and 372 x 604 pixels, respectively; hence, to make sure that the matriz size did not have an effect on the results, the PSP images were resized to 572 x 929 pixels, keeping the constrain aspect ratio.

Subtraction was conducted in EMAGO® / advanced 5.0.12 software (Oral Diagnosis Systems, Amsterdan, the Netherlands). Three methods of DSI were used: linear, logarithmic and advanced subtractions. The first and the second subtraction followed the normalization of the density of the two images (gamma correction command), the creation of a geometric corrected image called reconstruction image and the subtraction procedure. The third method is a combination of reconstruction, gamma correction and linear subtraction, i.e., the interactive software which transposed the two images, translated one image with respect to the other, automatically calibrates the gray levels, and then performed a subtraction in real time. Even with control of the exposure geometry, it was found necessary to perform subpixel translations and small rotation of the images before they were subtracted.

Image analysis

The data comprised eight digital radiography images of each tooth (80 remineralized approximal caries lesions and 20 control demineralized lesions): DCR and linear, advanced and logarithmic DSI with both systems (CMOS and PSP). In total, 800 digital images were coded and randomly organized and transported in slide presentations (Microsoft Office Power-Point 2003® software, Microsoft Corp., Redmond, WA). DCR were organized so that the corresponding paired images could be viewed side by side. The presentation was viewed on a 17 inch monitor (Sansung) with a resolution of 1024 x 768 pixel and a grayscale of 0-255, in two sections, by 5 radiologists. No adjustment of contrast and brightness was performed by the examiners. Each presentation was viewed on separate occasions to reduce examiner fatigue. All images were interpreted two times at least 2 weeks apart for intra-examiner reliability. Written and verbal instructions were given and samples images were shown of each modality to familiarize them with the types of images to be evaluated.

The first DCR displayed on the monitor was recorded by the examiners as follows: 1 = with demineralization, 0 = without demineralization. If it was decided that demineralization was present, the second image appears and as all methods of subtractions images the following decisions was made: 1 = increased intensity or 0 = no change. In the subtraction images, intensity increased (white areas) was interpreted as an indicator of the presence of mineral gained. The examiners were unaware of the ratio between treatment teeth in the sample.

Statistical analysis

The Ca/P_i analysis and the placement of the teeth on the remineralization solution [Ricketts *et al.*, 2007] was the validation for the true state of mineral gained in the approximal surface. Diagnostic accuracy was evaluated by the parameters of sensitivity and specificity. For the evaluation of accuracy differences between the methods of images and different remineralization protocols, McNemar's test was used to test this parameter. To evaluate intra and inter-examiner reliability Kappa statistics were calculated.

Results

Figure 1 shows the DCR side by side and the corresponding linear, advanced and logarithmic DSI of a remineralized tooth. The white area seen in the DSI indicates that the radiolucency of this area has decreased, most likely due to the deposition of minerals from the artificial saliva and fluoride solution. From the linear and logarithmic subtractions, it is clear that mineral gain has taken place, as the image on the approximal surface shows evidence of brighter subtraction shadow; however, the advanced subtraction and DCR did not demonstrate an easer visible intensity increase.

The demineralization diagnostic accuracy of DCR taken with PSP and CMOS systems was compared, there was a statistically significant difference ($p=0.0011$) between them, with a sensibility of 64% and 70%, respectively. The outcome of the remineralization evaluation is summarized in table 1, the sensitivity, specificity and accuracy values of the DCR and three methods of DSI with both systems (CMOS and PSP) are listed. The overall diagnostic accuracy of the methods in the detection of remineralization was higher for the CMOS system, statistically significant, in all four methods of evaluation, and the linear subtraction images were statistically more accurate than DCR, advanced and logarithmic DSI, by McNemar's test. In addition, the PSP system also demonstrated higher values of accuracy, statistically significant when the linear DSI were assessed.

The overall intra-examiner Kappa value was 0.62 (0.48 – 0.78) for CMOS and 0.43 (0.26 – 0.60) for PSP systems, while the inter-examiner was 0.34 (0.15 – 0.55) and 0.32 (0.16 – 0.51) when the demineralization diagnostic accuracy was assessed. While the intra and inter-examiner reproducibility, for mineral changes diagnosis, was found to be 0.50 (0.44 – 0.56) and 0.35 (0.28 – 0.39) for the CMOS system, and 0.60 (0.56 – 0.63) and 0.45 (0.40 – 0.52) for the PSP system, respectively.

Two different protocols and periods of remineralization were used, in a total of four groups, and it was noticed that the fluoride protocol obtained statistically significant higher values of ascertain answers, indicating that the use of F solution enhanced the diagnostic accuracy of mineral changes, independent of the method of image used, by McNemar's test (Table 2).

Discussion

The null hypothesis was not rejected; viewing paired DCR side by side and subjectively interpreting them for remineralization was less accurate then DSI. Thus mineral changes in approximal enamel are likely to be detected by subtraction images before they would by viewing paired radiographs side by side. The same outcome was achieved by Ricketts *et al*. [2007] when assessing occlusal demineralization progression, where subtraction radiography was found to be more accurate than visual assessment of paired digital images. Wenzel *et al*. [1992] also compared intensity increase as a sign of occlusal caries in subtraction radiography with the conventional radiolucency in film radiographs; however it was concluded that although the subtraction method did not provide a higher sensitivity, the intensity increase could be trusted more than traditional radiolucency as a sign of dentinal lesion.

Small alteration within enamel may be difficult to detect because of the complexity of structures projected over the area of interest, however removing this “structures” or “anatomic” noise by means of subtraction revealed clearly the radiographic density changes. In this study, a sensitivity rate of 57% and 46% was achieved on linear subtraction images assessment with CMOS and PSP systems, respectively, yielding a statistically significant diagnostic improvement of mineral changes compared to the other methods evaluated, being a suitable alternative in the monitoring of mineral changes of approximal caries-like lesions. Similar results have been demonstrated convincingly that subtraction of dental radiographs has the capability of providing detailed radiographic density changes within enamel and dentin that could not be discerned by visual comparison [Halse *et al.*, 1990]. Additionally, Maggio *et al.* [1990] claimed that the regression of carious lesions in extracted teeth can be evaluated using digital subtraction images, whereas increases in radiodensity or radiolucency have been detected in the deepest parts of carious lesions, depending on the conditions under the teeth were incubated.

Time-lapse radiographs were made using an orienting device that fixed the position of tooth, x-ray sensor / plate, and x-ray tube collimator. However, when standardization of projection geometry is provided, still further image manipulation is necessary to correct minor error. However, a complete lost of noise, unchanged anatomical structures, is difficult when comparing approximal surfaces. Logarithmic digital subtraction images have been pointed as a valuable technology, being either better or as good as linear subtraction [Haiter-Neto *et al.*, 2005]. However, in this study, it failed to reveal significantly superior on the diagnosis of mineral changes. The enhancement of structural noise is one of the characteristics of the logarithmic subtraction and it may be the cause of less accuracy compared to linear subtraction, i.e. that it made the actually mineral changes diagnosis less clear for the examiners causing misinterpretation of the logarithmic DSI, disabling to correctly discriminate between actually mineral changes and structural noise (Fig. 2). It is important to notice that with these standardization problems of radiographs the results from this *in vitro* study simulate clinical trials where providing standard projection geometry is even more difficult. Clinical studies are needed to achieve a better knowledge of the performance of subtraction images in mineral changes diagnosis, especially logarithmic subtraction.

A comparison of the diagnostic performance on enamel de and remineralization of both systems (CMOS and PSP) was assessed, and CMOS system performed better independently of the method used, digital conventional radiography or subtraction images. Very recent investigations have reported on caries diagnosis using the CDR Wireless® and Digora OpTime® methods. In perceived depiction of approximal dental caries, it was demonstrated that CMOS sensor provided a comparable diagnostic accuracy to charge-couple device (CCD) detector (Kitagwa *et al*., 2003) and to conventional film (Castro *et al*., 2007). In another study along the same track, comparing caries diagnostic accuracy, no significant difference was found between PSP plate and CCD-based sensor system (Hintze, 2006). A point that needs particular attention is that it was subjectively noted that CMOS system provided images with higher contrast and with more differences of gray level distribution between the two images used to be subtracted, that was caused by uncontrolled variation of the battery level and signal strengths. However, to suppress these problems the gray level distribution of the first image was used as reference to modify the gray level distribution of the second image by the "gamma correction" command of the software. But it is true that some valuable diagnostic information could become suppressed during digital image formation and disturbing information or noise could have been introduced.

Although there is a clear advantage of subjective methods they have limitations which are the variability of the lesions and the individual judgment of the investigator. However, in this study care was taken to eliminate other sources of interference. All images were prepared and manipulated by one author (J.A.B.C.) and viewed by other examiners. They were displayed for evaluation in the same 17″ computer monitor, with similar resolution / matriz size and stored in their original format with no compression [Wenzel, 2006]. Also, the images were displayed at the same image sizes. Haak *et al*. [2003] have concluded that image sizes with a display ratio of 1:1 and 1:2 resulted in better diagnostic validity than those with a ratio of 1:7. Although care was taken to eliminate differences of the matriz size between the two systems (CMOS and PSP), Prapayasatok *et al*. [2006] have concluded that images presented as a PowerPoint slide with different resolution settings did not have significant difference in diagnostic accuracy of caries lesions. It is possible that the whole process of manipulation and interpretation of the images could affect the diagnostic accuracy and reproducibility. The impact of this requires further investigation so does the effect of minor alterations in the angulation of the X-ray beam.

Observer agreement is often used as a method of assessing the reliability of subjective classification or assessment procedures. The reliability of tested analysis systems, CMOS and PSP, has been assessed, demonstrating levels of substantial and moderate intra-examiner agreement, for demineralization diagnostic accuracy, respectively. The opposite value of agreement was obtained for mineral changes diagnosis. Inter-examiner reliability on demineralization diagnosis, showed fair agreement for both systems, while the mineral changes diagnosis showed fair and moderate agreement for CMOS and PSP system, respectively. Interestingly, observers were able to assess demineralization correctly with the CMOS system, but express less confidence with this system regarding their assessment of mineral changes on enamel surface. Also, future research is required to investigate whether the fair inter-examiners agreement could have an influence on the outcome of this research.

The present study demonstrated that remineralization protocols, independent of the use of F solutions or treatment period, resulted in an increased in radiopacity, obviously caused by mineral gain. In relation to the appearance on the monitor, this resulted in an intensity increase in both DCR (less radiolucency) and DSI (a brighter area). However, the use of F solutions was able to spot the mineral changes correctly more often than they did with the saliva solution alone, i.e., that protocol B provided higher values of diagnostic accuracy, tending to make mineral changes more obvious, and it is also in accordance with the higher values of Ca an P_i analysis reduction, in the artificial saliva, found in this protocol. Also, the longer period of time provided slightly lower values of accuracy; future studies with longer and variables periods of time can be valuable to prove this conclusion.

In order to remineralize early lesions (demineralized enamel) before they cavitate, early detection and quantification of white-spot lesions are very important. With these methods of digital radiography, the results showed that they were able to detect remineralization therapies of early enamel lesions, being useful devices for longitudinal assessment of mineral changes in the enamel. However, further studies are needed, where there are no problems that the true-positive diagnoses are outweighed by false-negative diagnoses. In this study sensibility higher than 50% was only achieved by linear, advanced and logarithmic subtraction with the CMOS system.

Within the limitation of this study, it was concluded that (a) CMOS was more accurate than PSP on the diagnosis of artificial caries-like lesions and mineral changes monitoring; and (b) linear digital subtraction image can be a valuable method to disclose an intensity increase, as a result of mineral gain. However, further investigation is needed to identify an imaging system or enhancement mode to improve the detection and monitoring accuracy of incipient approximal caries lesions.

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Legends

Figure 1.

A sequentional set of images of DCR before and after remineralization, and linear, advanced and logarithmic DSI, respectively from left to right, with CMOS system (on the top) and PSP system (on the bottom) of the same tooth.

Figure 2.

Logarithmic subtraction image of a remineralized tooth showing the mineral gain (white arrow) and structural noise (black noise)

Figures

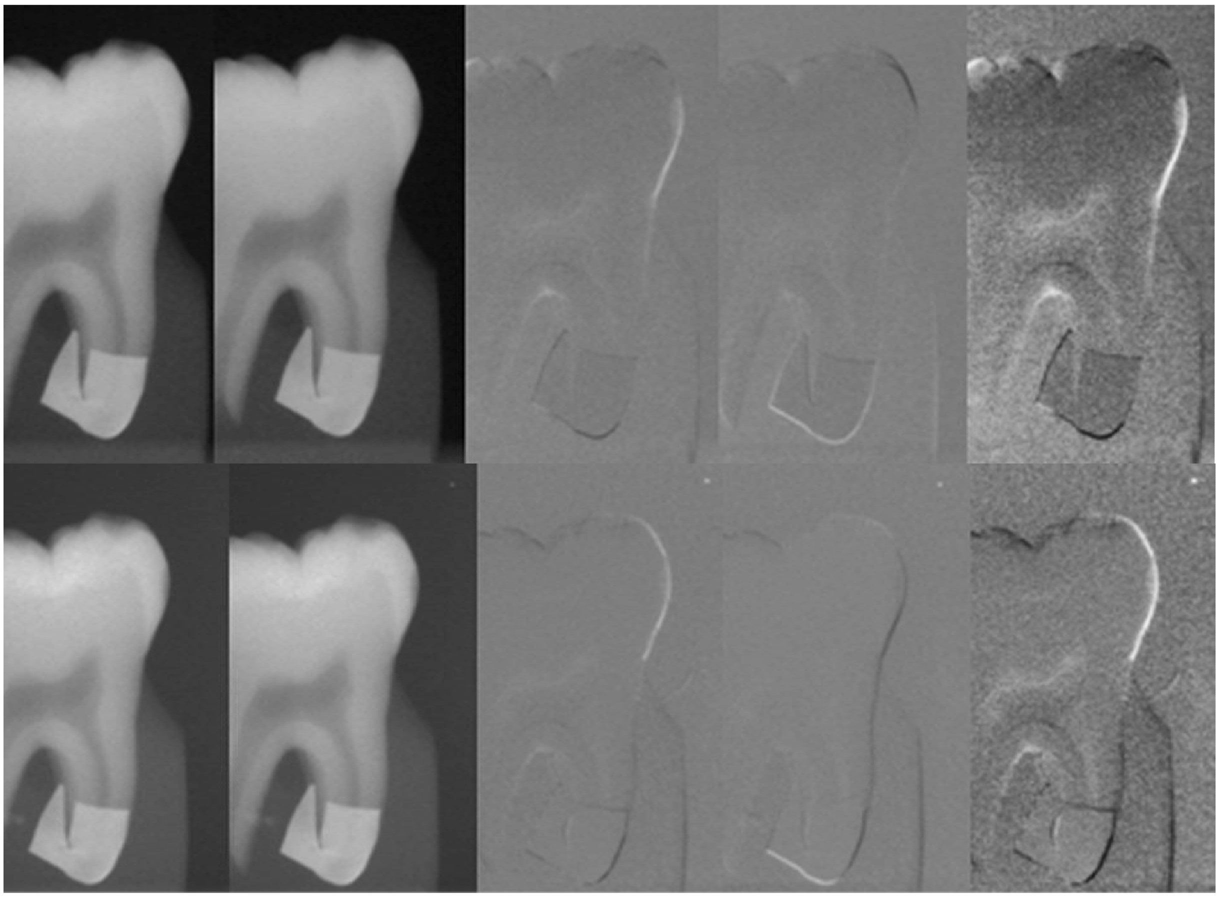

Figure 1

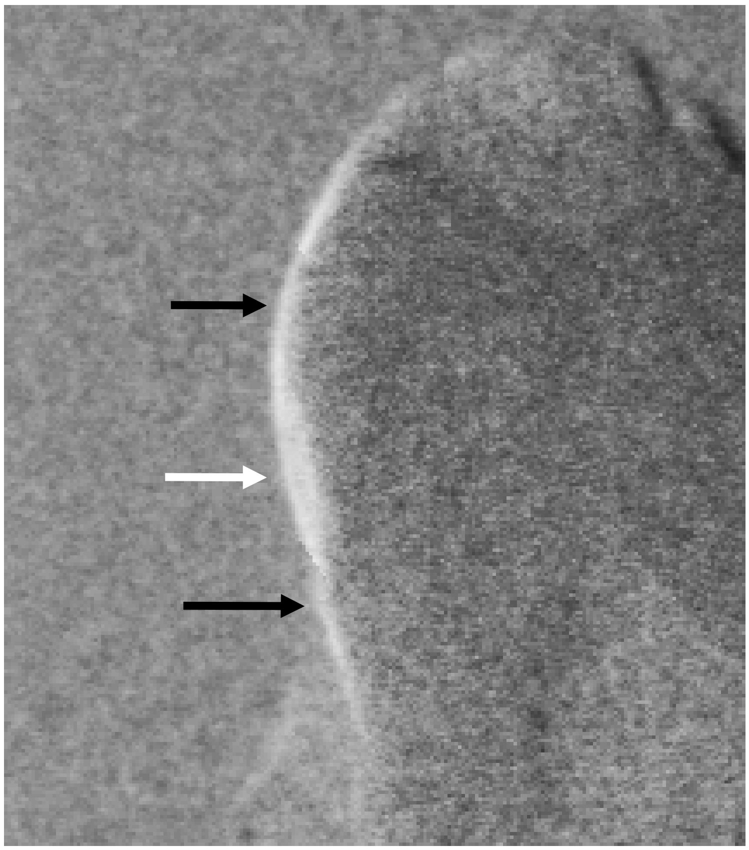

Figure 2

Tables

Table 1. Sensibility, especificity and accuracy values of mineral changes diagnosis with both systems and four methods of images

System	Image method	Sensibility	Specificity	Accuracy
CMOS	DCR	0.451	0.795	0.520
	Linear DSI	0.576	0.735	0.608
	Advanced DSI	0.516	0.750	0.563
	Logarithmic DSI	0.543	0.690	0.573
PSP	DCR	0.292	0.900	0.414
	Linear DSI	0.465	0.770	0.526
	Advanced DSI	0.365	0.680	0.428
	Logarithmic DSI	0.412	0.785	0.487

Table 2. Accuracy values of mineral changes diagnosis comparing protocol A (continuous remineralization) and protocol B (continuous remineralization + fluoride treatment) after 4 and 8 weeks

Protocols	Period (weeks)	Accuracy
A	4	43.3%
	8	42.5 %
B	4	48.7 %
	8	46.5 %

CAPÍTULO 4

TITLE PAGE

Noise in linear and logarithmic subtraction images made from pair of images with CMOS sensor and PSP plate

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SHORT TITLE: **Noise in subtraction images**

ABSTRACT

Objectives: To compare noise and reproducibility in linear and logarithmic digital subtraction images (DSI) produced from two digital radiography systems in three different regions of interest (ROI), varying size and unchanged structures.

Methods: Eighty pairs of digital radiography images were obtained with photostimulable storage phosphor (PSP) plate and complementary metal oxide semiconductor (CMOS) sensor. Linear and logarithmic digital subtraction images (DSI) were produced using EMAGO®/Advanced software with repetitions. Three ROI were selected with two different sizes and in two different unchanged structures, and the mean shades of gray and the standard deviation (SD) of the histogram were assessed. Afterwards, statistical analysis was performed.

Results: All subtraction images from both systems of digital radiography were reproducible, except logarithmic subtraction using CMOS sensor in ROI of 10.000 pixels. Comparing CMOS sensor and PSP plate, both values of mean shades of gray and SD, it was statistically significant different by Mann Whitney's test, PSP plate presented values of higher mean shades of gray, closer to 128, and lower values of SD. It was also statistically significant the differences between ROI of different size and unchanged structures.

Conclusions: Subtraction images from PSP plates had statistically less noise than images produced from CMOS sensor, however both systems was reproducible when creating subtraction images. Cautions have to be taken when assessing quantitatively ROI with different sizes and in different structures.

Key Words: subtraction technique; digital radiography; noise

INTRODUCTION

Quantitatively evaluation differences occurring over a time interval on digital radiographs have been done by digital subtraction technique.[1] However, a perfect geometrical match and exposure conditions is required. Some basic characteristics of the subtraction software are to present algorithms for automatic registrations of dental radiographs to correct these differences between two images.

The correction of projection geometric differences can be done by manual registration techniques that are usually based on landmarks which are marked in both images to be registered. Using deformation algorithms, the corresponding points are mapped exactly onto each other while the others are interpolated based on the triangulation of the spatial domain or on energy minimization models. Thereby, the results registration is extremely dependent on the actual positioning of the corresponding points and therefore, image warping is highly observer-dependent. On the other hand the correction of gray levels between the two images is performed automatically by the software.

When two radiographs are recorded with controlled projection angles and thereafter subtracted, theoretically all unchanged anatomical background structures are cancelled, and these areas displayed in a neutral gray shades (pixel value = 128) in the subtraction image, while regions that have changed between the radiographic examination are displayed in darker (pixel value < 128) or lighter (pixel value > 128) shades of gray. The result is an image highlighting the difference between the subtracted radiographs against a relatively uniform background. However, subtraction images can contain gray-level variation which is independent of any changes produced from real differences. These “accidental” differences can thus interfere with diagnostic accuracy and it is called noise. It should be possible, in quantitative digital subtraction to detect any change in pixel value which truly originates from minute change in the object by eliminating the variation in the sensitivity of the pixels (noise).[1] This noise can thus be quantified by using the SD of the histogram defining the distribution of gray shades in the subtraction image.[2] The more shades of gray in the histogram, more noise in the subtraction image, and the larger the SD.[3]

Based on these, the aim of this study is to compare noise and reproducibility in linear and logarithmic DSI produced from two digital radiography equipments: DIGORA OpTime® (Orion Corp./Soredex, Helsinque, Finlândia) PSP plate; and CDR Wireless® (Schick Technologies, NY, USA), CMOS sensor.

MATERIALS AND METHODS

A set of 80 pairs of *in vitro* radiographs taken before and after a remineralization protocol on human teeth was selected for this study. The teeth were mounted in individual acrylic resin base, except the crowns, giving the teeth a proper stability to take the radiographs; a peace of aluminum was also inserted in the acrylic base to be used as reference points during their alignment. The radiographs were taken using two digital radiography equipments: DIGORA OpTime® (Orion Corp./Soredex, Helsinque, Finlândia) photostimulable storage phosphor (PSP) plate; and CDR Wireless® (Schick Technologies, NY, USA) complemetary metal oxide semiconductor (CMOS) sensor.

In EMAGO® / advanced 3.43 software (Oral Diagnosis Systems, Amsterdan, the Netherlands) the pair of images were manipulated. The first step of the subtraction procedure was to modify the gray level distribution of first image using the gray level distribution of second image as reference, i.e. to calibrate the gray levels of all pixels in the full image between the first and the second radiograph by the gamma correction command. An alignment of the images was also performed by the reconstruction command to correct small geometric misalignments. Then, two methods of DSI were obtained: linear and logarithmic. The later method is identical to linear subtraction, except for the fact that it enhances small differences, but at the same time noise and contrast also increases. To estimate the reproducibility of the systems, these steps were completed twice, providing four subtractions for each pair of images.

Afterwards, three regions of interest (ROI) consisting of two different sizes were set on two different unchanged structures by the rectangle command also in EMAGO® software. The unchanged structures were: the crown of the tooth and the aluminum positioned in the resin base. The ROI consisted of 1.200 and 10.000 pixels (Figure 1-3). Then mean gray shades and standard deviation (SD) values of the pixels in each ROI were determined by the histogram command. The repeated subtractions were assessed at the same time to lower the observer error.

Statistical Analysis

The mean pixel value and SD of the histogram defining the distribution of gray shades in the subtraction image was used as the statistical parameter in the comparison of the homogeneity of the images created from two digital radiography systems as described previously.[3] Mann Whitney's test was performed to evaluate differences between the means and SD in subtraction images from both systems CMOS sensor and PSP plate. A comparison of the difference between the ROI were also performed by Friedman's test. The difference between double measurements was evaluated by Wiloxon's pair rank test, probabilities smaller than 0.05 were regarded as statistically significant.

RESULTS

Tables 1 and 2 show median and range of the mean gray shades and SD (from linear and logarithmic DSI) in the two digital systems and three evaluated ROI. Both methods of DSI showed similar outcomes. Both systems, CMOS sensor and PSP plate obtained good repeatability of mean gray shades and SD values, i.e., there was not a statistical significant difference between the mean pixel value and SD of all ROI in repeated subtracted images; except for a ROI of 10.000 pixels in logarithmic DSI with CMOS sensor, both mean gray shades and SD were statistically different by Wilcoxon's test ($p = 0.0002$ and 0.0042, respectively). Comparing subtractions images obtained with CMOS sensor and PSP plate, there was a statistically significant difference ($p < 0.05$) with median values closer to 128 and lower SD in the subtraction obtained from PSP plate.

The different size of ROI and different unchanged structures with the same size were also statistically significant different in all evaluations values ($p < 0.05$).

DISCUSSION

To obtain valid quantitative data by digital subtraction radiography, the methodological error underlying the results should be determined and corrected, so true changes can be distinguished from changes caused by method error. Quantification of radiographic density can be done either absolutely by using reference objects, or relatively, by comparing density changes in defined units with the changes in areas affected by hard tissue changes.[4,5] Even though corrections for differences in the two radiographs may be performed, it is still needed to take the level of noise in the image into account. The ROI assessed in this study had as an aim to establish the variation that can occur in test areas when different structure and size of the area and also different digital radiographic system are used.

Since the mean gray shades of the subtraction histogram did not reflect the closeness of the repositioned images to the original image, the SD of the subtraction histogram was also considered to measure the ability of the software to quantitatively perform subtractions; therefore, a comparison of the SD of the three ROI and the two digital systems were performed.[2] In theory, a homogeneous DSI will consist of pixels with little variation in shades of gray, i.e. in ROI that had not change during the time interval evaluated, the values of mean gray shades is 128 and the SD is 0, with no noise.

However, these values were not obtained in either of ROI used; i.e differences are proved by comparing two ROI of the same size in unchanged anatomical structures (the crown of the teeth and the aluminum inserted in the resin base). Mean gray-level pixel value difference was observed in this ROI, which were obtained under the same exposure conditions. Longitudinally obtained radiographs present some inherent noise that could possible explains these results, being difficult to characterize explicitly because there are so many non-linear factors that can lead to inaccuracies of this sort. Some non-linear factors include changes in radiographic density caused by beam hardening, radiation scatter and inherent to various components of the imaging system.[6] The smallest SD values were obtained when PSP plate was used and ROI with areas of 1.200 pixels located in the crown. It can be concluded that PSP plate was more ideal for the subtraction procedure; and it is suggested to use as reference, ROI located in structures with less density and smaller areas to prevent inherent variations on the results. It has also been shown that although ROI size and shape consistency in longitudinal studies are important in density analyses, small variations has minimal impact.[7] In spite of this conclusion, higher variations of the ROI size can be a critical factor in the measurement of noise in test regions by the histogram command.

The quality of adjustment can be affected by the degree of precision in the positioning of the landmarks. Therefore, the registration depends on the observer placing the landmarks in both images. However, in this study, the two digital radiographically systems were reproducible, this indicates that reliable subtraction can easily be obtained by the two digital imaging systems used. However, the logarithmic DSI obtained from radiographs taken with the CMOS sensor have to be used carefully with ROI consisting in 10.000 pixels.

The ability to accurately quantify early changes with subtraction histogram is valuable and it is suggested, but it is important to take into account characteristics that can make variations from the basic theory of the mean gray shades and SD of the histogram. Cautions should be taken for the precise quantification of differences occurring in a period of time on subtraction images.

ACKNOWLEDGEMENTS

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FIGURES LEGENDS

Figure 1.

Description of ROI A (on the crown consisting of 1.200 pixels) and performed histogram.

Figure 2.

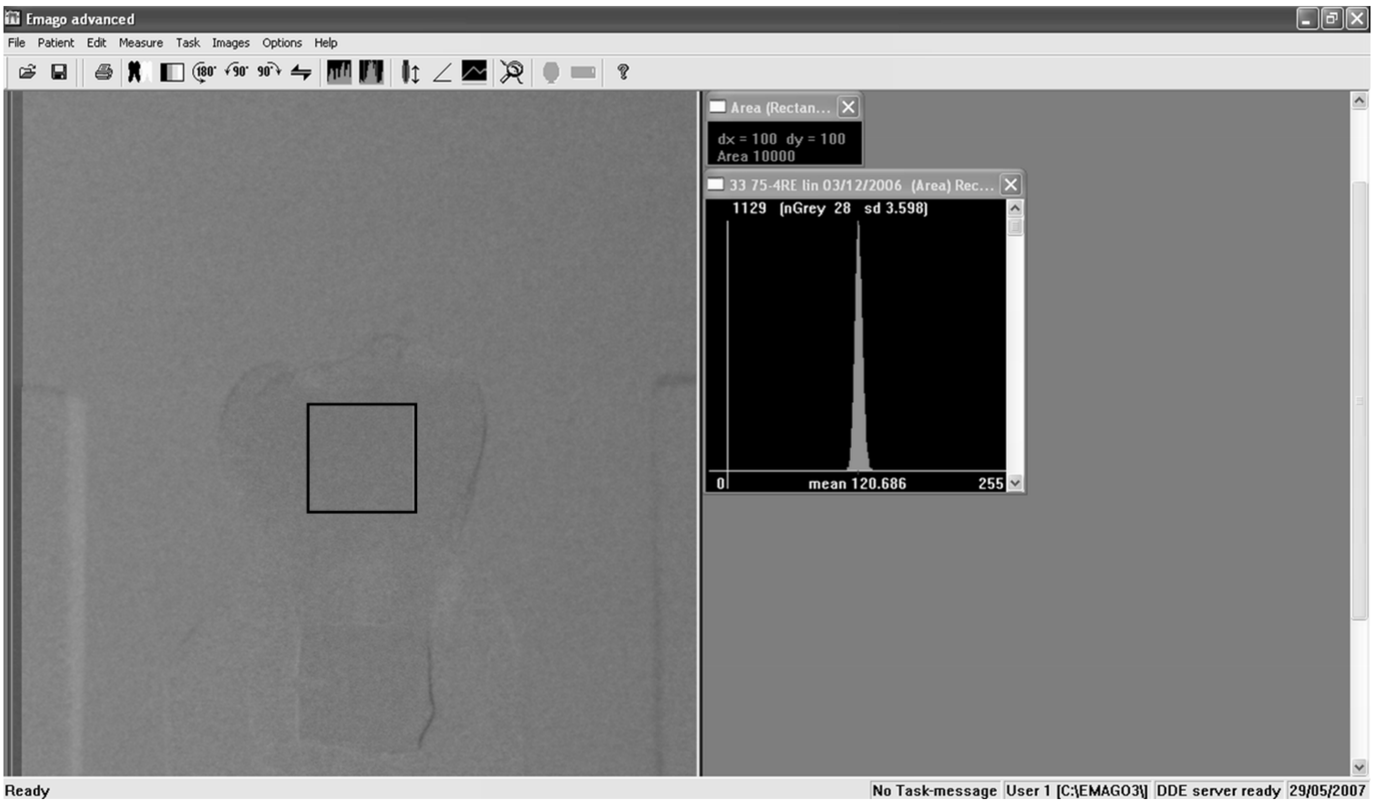
Description of ROI B (on the crown consisting of 10.000 pixels) and performed histogram.

Figure 3.

Description of ROI C (on the aluminum consisting of 1.200 pixels) and performed histogram.

FIGURES

Figure 1.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mean 120.686
255
Ready
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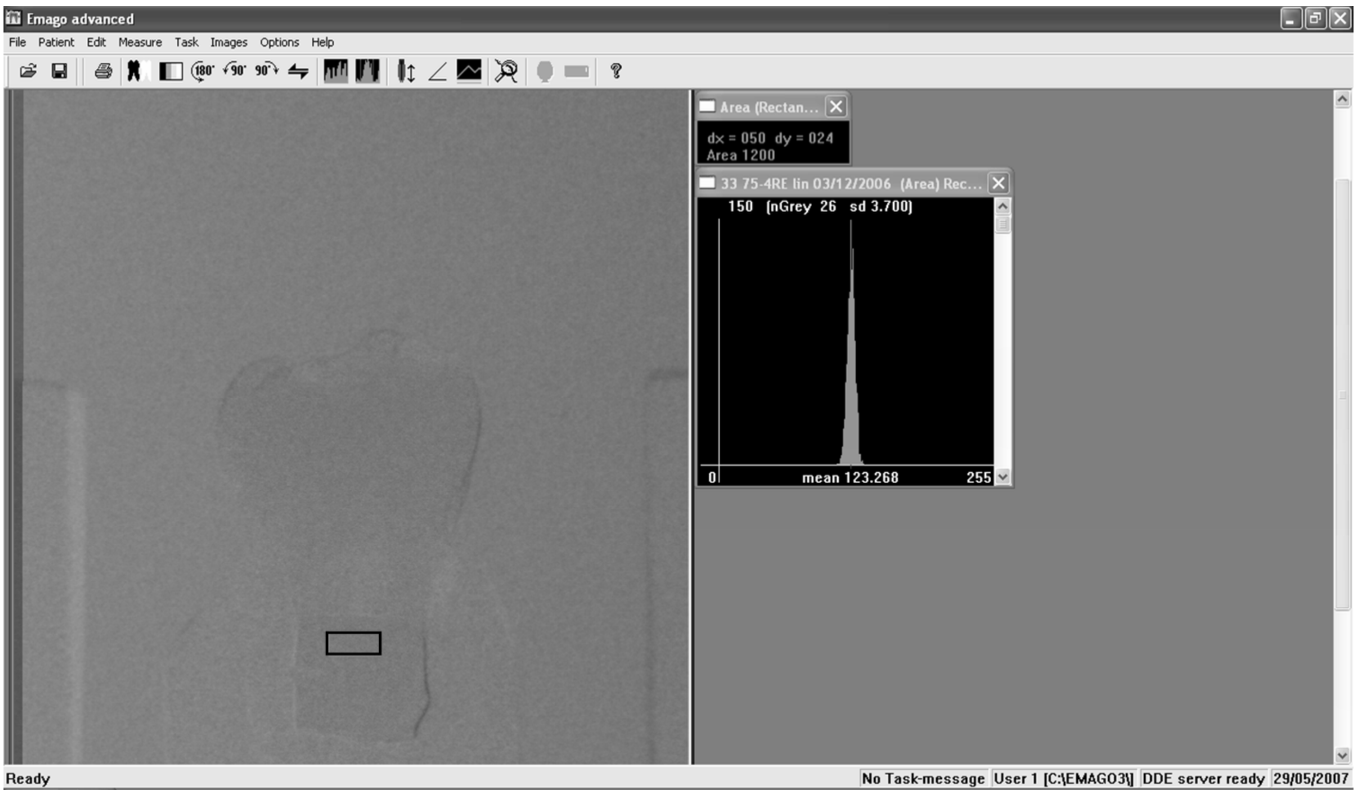
User 1 [C:\EMAGO3\]
DDE server ready
29/05/2007

Figure 2.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User 1 [C:\EMAGO3\]
DDE server ready
29/05/2007

Figure 3.

TABLES

Table 1 Median of the mean grey shade (range) and standard deviation (range) for the histograms in the linear subtraction images obtained with two digital radiography systems in three different regions (A – on the crown with 1.200 pixels; B – on the crown with 10.000 pixels; and C – on the aluminum with 1.200 pixels).

Values	System	Areas			P-value*
		A	B	C	
Mean grey shade	**CMOS sensor**	119.5 (115.6-141.6)	120.8 (116.0-125.0)	123.5 (118.3-128.1)	0.000
	PSP plate	125.3 (120.9-128.6)	125.5 (121.6-129.1)	126.1 (122.3-132.5)	0.000
Standard deviation	**CMOS sensor**	3.6 (3.2-3.9)	4.0 (3.5-4.7)	3.9 (3.3-5.1)	0.000
	PSP plate	2.2 (1.9-3.0)	2.3 (2.1-3.2)	2.2 (2.0-3.3)	0.000

* P-value were calculated using Friedman test (P<0.05).

Table 2 Median of the mean grey shade (range) and standard deviation (range) for the histograms in the logarithmic subtraction images obtained with two digital radiography systems in three different regions (A – on the crown with 1.200 pixels; B – on the crown with 10.000 pixels; and C – on the aluminum with 1.200 pixels).

Values	System	Areas			P-value*
		A	B	C	
Mean grey shade	**CMOS sensor**	95.0 (81.3-115.5)	99.2 (85.2-119.0)	112.0 (89.1-132.3)	0.000
	PSP plate	119.6 (100.2-133.3)	120.1 (103.2-135.3)	123.4 (104.8-150.7)	0.000
Standard deviation	**CMOS sensor**	13.4 (11.3-15.9)	15.8 (13.5-18.0)	16.3 (13.4-19.1)	0.000
	PSP plate	9.9 (8.2-10.9)	10.5 (9.2-14.2)	10.3 (9.0-16.4)	0.000

* P-value were calculated using Friedman test (P<0.05).

CONCLUSÃO

A partir dos dados estudados pelo presente trabalho, verificou-se que:

A - Os métodos de dureza do esmalte e microscopia de luz polarizada não foram capazes de detectar o ganho mineral observados pelas dosagens bioquímicas de Ca e P_i, durante os tratamentos de remineralização dentária. Entretanto, por meio das imagens de subtração radiográfica digital foi possível observar este ganho mineral, que mostra poder ser um método para o monitoramento da remineralização de lesões de cárie interproximal incipiente.

B - As dosagens bioquímicas de Ca e P_i foram fortemente correlacionadas, indicando ser o método mais sensível para o monitoramento quantitativo de mudanças minerais no esmalte dentário. E a determinação da densidade por meio do histograma em regiões de interesse, pode ser uma alternativa para o monitoramento das mudanças minerais em imagens de microscopia de luz polarizada.

C - O sistema CMOS obteve maiores valores de acurácia no diagnóstico de desmineralizações e no monitoramento de mudanças minerais. E o método de subtração radiográfica digital linear foi o melhor método para a detecção de um aumento de intensidade como resultado do ganho mineral no esmalte dentário.

D - Imagem de subtração radiográfica digital obtida a partir de pares de imagens com a placa PSP contém menor quantidade de ruído comparando imagens obtidas a partir do sensor CMOS, porém as subtrações com os dois sistemas de radiografias digitais são reprodutíveis. A avaliação quantitativa a partir do histograma, comparando estruturas e regiões de tamanhos diferentes não é confiável.

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ANEXO 1

COMITÊ DE ÉTICA EM PESQUISA

FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Radiografia digital e a técnica de subtração no monitoramento da desmineralização e remineralização do esmalte dentário**", protocolo nº **018/2005**, dos pesquisadores **FRANCISCO HAITER NETO e JULIANA ARAUJO BITTAR CORTEZ**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 03/05/2005.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Digital radiography and the subtraction technique for monitoring dental enamel demineralization and remineralization**", register number **018/2005**, of **FRANCISCO HAITER NETO and JULIANA ARAUJO BITTAR CORTEZ**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 03/05/2005.

Cinthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2

De: r.p.shellis@bristol.ac.uk
Assunto: Ms. No. 200712006, Caries Research
Data: Qui, Dezembro 13, 2007 12:20 pm
Para: bittarcortezja@fop.unicamp.br

MS: 200712006
Dear Dr. Bittar-Cortez,

Thank you for submitting your manuscript entitled "In vitro remineralization of artificial carious lesions assessed by subtraction images" to "Caries Research". It will now be submitted to review and we shall inform you as soon as possible of the decision reached by the editorial board. The manuscript reference number is 200712006. Please use this number on all correspondence about the manuscript, which should be sent to the "Caries Research" editorial office at the address listed below.

With kind regards,

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(Editor-in-Chief, Caries Research)
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