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BRUNO BOCHNIA CERCI

RUGOSIDADE DO ESMALTE DENTÁRIO COM DIFERENTES TEMPOS DE CONDICIONAMENTO ÁCIDO: ESTUDO COM MICROSCOPIA DE FORÇA ATÔMICA

CURITIBA

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Dissertação apresentada ao Programa de Pós-graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para a obtenção do Título de Mestre em Odontologia, Área de Concentração em Ortodontia.

Pós-graduando: Bruno Bochnia Cerci

Orientador: Prof. Dr. Orlando Tanaka

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TERMO DE APROVAÇÃO

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Rugosidade do Esmalte Dentário com Diferentes Tempos de Condicionamento Ácido: Estudo com Microscopia de Força Atômica

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RESUMO

Desde a introdução do condicionamento ácido do esmalte, muitas pesquisas investigaram a resistência adesiva, mas poucas analisaram a topografia de superfície do esmalte condicionado. Uma característica do esmalte dentário pouco estudada em detalhe é a rugosidade na escala microscópica. O objetivo deste estudo foi analisar quantitativa e qualitativamente a topografia da superfície do esmalte condicionado com ácido em diferentes tempos. Noventa e seis pré-molares superiores humanos extraídos, após a realização de profilaxia com pasta de pedra-pomes foram distribuídos aleatoriamente em 3 grupos (n=32): T0 (controle), esmalte não condicionado; T15, esmalte condicionado com ácido fosfórico 35% por 15s; T30, esmalte condicionado com ácido fosfórico 35% por 30s. Imagens tridimensionais da topografia do esmalte foram obtidas com microscopia de força atômica (MFA), em uma área de 30μmx30μm, na qual também foi realizada análise quantitativa de superfície obtendo-se as médias aritimética (Ra) e quadrática (Rms) da rugosidade, e a distância média entre 5 picos máximos e 5 vales máximos (Rz). Adicionalmente, fotomicrografias foram obtidas com microscopia eletrônica de varredura (MEV), em magnificações de 4000X, para ilustrar os resultados. ANOVA e o teste de comparações múltiplas de Games-Howell foram utilizados. As variáveis de rugosidade Ra, Rz e Rms apresentaram diferenças estatisticamente significantes para todos os grupos ($p<0.000$), com valores aumentando com o tempo de condicionamento. Este aumento da rugosidade foi maior de T0 para T15 quando comparados de T15 para T30. As alterações da superfície do esmalte de T15 para T30 ocorrem principalmente pelo aumento da altura (Rz) e aprofundamento do centro do prisma. Conclui-se que a rugosidade do esmalte dentário aumenta progressivamente com o tempo de condicionamento ácido de até 30s.

Palavras-chave: Topografia, Esmalte Dentário, Condicionamento Ácido Dentário, Microscopia de Força Atômica, Microscopia Eletrônica de Varredura.

INTRODUÇÃO

O advento do condicionamento ácido ao esmalte dentário, na década de 1950 (Buonocore 1955), tornou possível a adesão em Odontologia. O condicionamento ácido causa uma descalcificação seletiva aumentando a energia livre de superfície, a porosidade do esmalte e a área de superfície (Beech e Jalaly 1980). A adesão ao esmalte é dependente da capacidade das resinas em penetrar entre os prismas e cristais de apatita (Shinchi et al. 2000), resultando em retenção micromecânica. O encapsulamento dos cristais de apatita pela resina infiltrada forma *micro-tags* (Van Meerbeek et al. 2003) e constituem a camada híbrida a qual promove um mecanismo de nanoretenção entre a estrutura dentária e o material resinoso (Hannig et al. 2002; Nakabayashi e Pashley 1998). Os *micro-tags*, provavelmente, contribuem mais para a efetividade da adesão do que os *macro-tags* que preenchem as periferias dos prismas (Van Meerbeek et al. 2003).

As características de retenção da superfície do esmalte condicionado dependem da composição química do esmalte, do tipo e concentração do ácido utilizado e do tempo de condicionamento (Carstensen 1992; Legler et al. 1989; Powers et al. 1997). Alguns estudos demonstraram que uma variação de 15 a 90 segundos de condicionamento com ácido fosfórico 35-37% não apresenta impacto significativo sobre a resistência adesiva ao cisalhamento de *brackets* ortodônticos (Barkmeier et al. 1987; Triolo et al. 1993; Wang e Lu 1991).

Apesar de muitos estudos realizados para a caracterização da superfície do esmalte condicionado por ácido (Poole e Johnson 1967; Marshall et al. 1975

Silverstone et al. 1975; Galil e Wright 1979a,b; Oliver 1987; Gardner e Hobson 2001; Hobson et al. 2001, 2002; Hobson e McCabe, 2002), a maioria utilizou microscopia eletrônica de varredura (MEV) e, portanto, fornecem apenas dados qualitativos da topografia do esmalte dentário. Os padrões de desmineralização do esmalte observados com MEV por Galil e Wright (1979) foram classificados em cinco tipos: 1) Dissolução preferencial da região central dos prismas resultando numa aparência de "favos de mel"; 2) Dissolução preferencial da periferia dos prismas resultando numa aparência de "paralelepípedo"; 3) Combinação dos tipos 1 e 2; 4) Superfície do esmalte porosa semelhante a mapas ou redes; e 5) Superfícies planas e suaves.

A rugosidade de superfície é tradicionalmente expressa numa medida que representa a topografia média e macroscópica da superfície como um todo. Detalhes microscópicos podem ser negligenciados por limitações do instrumento utilizado. O microscópio de força atômica (MFA), com alta resolução lateral e vertical, permite a exploração desta rugosidade em baixa escala que é pouco influenciada por componentes macroscópicos como as ondulações da superfície, as quais são menos relevantes ao estudar processos envolvendo partículas microscópicas (Mendez-Vilas et al. 2007).

O efeito que a rugosidade de superfície exerce sobre a resistência adesiva não é completamente entendido (Gardner e Hobson 2001). Porém Eick et al. (1972) afirmaram que o aumento da rugosidade produz maior área de superfície e caso ocorra um favorável contato entre o adesivo e o esmalte, o aumento da área de superfície levará ao máximo molhamento e conseqüente melhora da força de adesão.

Desde a introdução do condicionamento ácido do esmalte, muitas pesquisas investigaram a resistência adesiva, mas poucas analisaram a topografia de superfície do esmalte condicionado (Hobson et al. 2002b). Uma característica do esmalte dentário pouco estudada em detalhe é a rugosidade na escala microscópica (Casas et al. 2008). Portanto, o objetivo deste estudo foi comparar e analisar qualitativa e quantitativamente as alterações da rugosidade do esmalte, filtrando-se as ondulações macrométricas, que ocorrem com o condicionamento ácido por 15 e 30 segundos.

MATERIAIS E MÉTODOS

Preparação da Amostra

Noventa e seis pré-molares superiores humanos íntegros, extraídos por indicação ortodôntica, provenientes do Banco de Dentes da PUCPR, foram armazenados em solução de cloramina T 0,5% por sete dias, e então armazenados em água destilada a 4ºC, trocada a cada sete dias, conforme ISO/TS 11405. Todos os procedimentos foram aprovados pelo Comitê de Ética em Pesquisa da Pontifícia Universidade Católica do Paraná (ANEXO I, p.47,48).

Os corpos de prova foram obtidos seccionando-se a face vestibular de todos os pré-molares, com disco flexível diamantado perfurado 7015 (KG Sorensen, Barueri, SP, Brasil), de modo a manter o terço médio da face vestibular paralelo ao plano de corte. Foi realizada profilaxia com taça de borracha e pasta de pedra-pomes e água por 10s, rinsagem com jato de água destilada por 20s e secagem por 20s com jato de ar. Os dentes foram distribuídos aleatoriamente em 3 grupos (n=32): T0 (controle), esmalte não condicionado; T15, esmalte condicionado com ácido fosfórico 35% (3M Dental Products Division, St. Paul, MN) por 15s, lavados por 20s e secados por 20s; T30, esmalte condicionado com ácido fosfórico 35 % por 30s, lavados por 20s e secados por 20s. Trinta corpos de prova de cada grupo foram analisados no microscópio de força atômica (MFA) e dois no microscópio eletrônico de varredura (MEV).

Microscopia de Força Atômica (MFA)

Todas as imagens foram obtidas com o MFA Shimadzu SPM-9500J3 (Shimadzu Co., Tóquio, Japão) utilizando-se uma ponta de contato piramidal de nitreto de silício

(Olympus, Tóquio, Japão) com força constante, em uma área de 30µmx30µm, amplitude vertical máxima (Z) de 5µm, freqüência de 1Hz, resolução 512x512, e *operating point* 2V.

Três imagens do terço médio da face vestibular do corpo de prova amostra foram selecionadas para realização da análise de superfície. Foi observado que a maioria das imagens, apresentavam dissolução compatível com o padrão de desmineralização tipo 1 (Galil e Wright 1979). Deste modo, utilizou-se somente este padrão, para evitar que a topografia macro-geométrica dos diferentes padrões prejudicasse a avaliação da micro-rugosidade, objetivo principal deste estudo. As imagens foram planificadas com o *software SPM Manager v2.11* da Shimadzu, e então aplicou-se a análise de superfície para a obtenção dos seguintes dados: Ra (média aritimética da rugosidade), Rz (distância média entre 5 picos máximos e 5 vales máximos) e Rms (média quadrática da rugosidade), e a análise de perfil para ilustrar os resultados.

Microscopia Eletrônica de Varredura (MEV)

Seis corpos de prova, sendo 2 de cada grupo, foram revestidos com 15 nm de ouro e armazenadas em um dessecador por 48 horas. Fotomicrografias em magnificações de 4000X foram realizadas com o MEV Jeol JSM 6360-LV (Tóquio, Japão) operado com 15 kV para ilustrar os resultados.

Análise Estatística

A média das variáveis Ra, Rz e Rms foi obtida a partir das 270 imagens, 3 de cada um dos 90 corpos de prova.

O teste de homogeneidade de variâncias de Levene indicou que os grupos apresentam variâncias heterogêneas para as três variáveis. Utilizou-se ANOVA e o teste de comparações múltiplas de Games-Howell para evidenciar as diferenças entre os valores médios das variáveis para os diferentes grupos. O teste de correlação de Pearson foi utilizado para correlacionar as variáveis de rugosidade.

RESULTADOS

MFA

A Tabela 1, p.19 descreve as variáveis Ra, Rz e Rms para os três grupos. Todas as variáveis apresentaram diferença estatisticamente significante entre grupos ($p<0.0000$). A rugosidade aumentou, não linearmente, com o aumento do tempo de condicionamento ácido (Figura 1, p.20).

As três medidas Ra, Rz e Rms apresentaram alta correlação entre si ($R>0.959$ e $p<0.0000$).

As imagens 3D do padrão tipo 1 de desmineralização do esmalte condicionado em T30 (Figura 2e, p.21) apresentaram uma maior remoção de mineral da região central dos prismas quando comparadas com T15 (Figura 2c, p.21).

MEV

O esmalte que foi realizado somente profilaxia com pedra-pomes apresentou uma superfície regular e eventuais riscos (Figura 2b, p.21), e o esmalte condicionado com ácido fosfórico 35% em T30 (Figura 2d, p.21) apresentou o padrão de desmineralização tipo 1 mais definido que o condicionado em T15 (Figura 2f, p.21).

DISCUSSÃO

A rugosidade é definida como o conjunto de irregularidades, ou, pequenas saliências e reentrâncias que caracterizam uma superfície e influem na resistência oferecida ao escoamento de fluidos, na qualidade de aderência e no brilho. Apesar da rugosidade micro-mecânica ser apontada como primordial para a obtenção de adesão eficiente ao esmalte dentário (Gwinnett e Matsui 1967; Buonocore et al. 1968), não se conhece precisamente quais características do esmalte condicionado estão envolvidas e em que escala de grandeza métrica ocorre a adesão.

Uma característica do esmalte dentário pouco estudada em detalhe é a rugosidade na escala microscópica (Casas et al. 2008). O MFA possibilita a expansão dos conhecimentos da microestrutura e o comportamento da desmineralização de tecidos, além de permitir o entendimento dos processos físico-químicos que ocorrem na superfície do esmalte dentário quantitativa e qualitativamente (Jandt et al. 2000).

Os padrões de desmineralização, quando analisados estaticamente, podem ser diferenciados e classificados (Galil e Wright 1979). A desmineralização não ocorre igualmente por toda a superfície do esmalte em relação ao tempo devido à orientação dos cristais de apatita, iniciando-se na interface entre o centro e as paredes dos prismas, desenvolvendo-se anisotropicamente ao longo do prisma (Wang et al., 2005). No presente estudo, o esmalte condicionado com ácido fosfórico 35% por 30 s (Figuras 2e,2f, p.20) revelou um padrão tipo 1 mais definido e aprofundado no centro do prisma do que por 15s (Figuras 2c,2d, p.20). Em concordância, Gardner e Hobson (2001)

utilizando ácido fosfórico 37% encontraram os padrões 1 e 2 para o tempo de 30 segundos mais definidos quando comparados a 15 segundos.

De acordo com estudo realizado por Carstensen (1992), a relação entre o padrão de desmineralização e a resistência adesiva ao cisalhamento indica que os padrões tipo 1 e 2 promovem máxima adesão. Adicionalmente, a longevidade da colagem é influenciada pelo padrão de desmineralização (Hobson et al. 2002a). Entretanto, Nakabayashi e Pashley (1998) sugeriram que a resistência adesiva entre resina e esmalte é o resultado da área transversal cumulativa dos *tags* de resina infiltrados no espaço do esmalte. Portanto, a exposição dos cristais seria mais importante do que padrões de desmineralização bem definidos (Hobson et al. 2002a). Orellana et al. 2008 sugeriram que a porosidade do esmalte é mais importante do que padrões de desmineralização definidos utilizando pioneiramente o método BET (adsorção de gás), no qual não encontraram correlação entre a área específica de superfície do esmalte condicionado com ácido e os diferentes padrões de desmineralização.

No presente estudo, o aumento da rugosidade em relação ao tempo de condicionamento ácido, apesar de progressivo não foi linear, aumentando em menor proporção com o tempo. Houve um maior aumento de todas as variáveis de rugosidade de T0 para T15, que de T15 para T30. No entanto, a quantidade de mineral removido no processo de desmineralização mostrou-se linear em relação ao tempo de condicionamento (Wang et al. 2005). Watari (2005) encontrou o mesmo comportamento para a espessura de esmalte condicionado com ácido fosfórico 2%

para tempos de até 3 minutos, encontrando rugosidades médias (Ra) com aumentos menores com o tempo.

As medidas de rugosidade Ra e Rms representaram, respectivamente, a média aritimética e a quadrática do desvio das rugosidades em relação ao perfil médio da superfície. A medida Rms é, portanto, mais sensível para valores extremos (Gadelmawla et al. 2002), os quais estão representados na medida de rugosidade Rz. Apesar da medida Ra ser muitas vezes utilizada como o único indicador da rugosidade, esta não é capaz de indicar a profundidade das irregularidades (Eliades et al. 2004), pois picos e vales são registrados da mesma maneira (Whitehead et al. 1996). Entre os grupos observados, as medidas Rz e Rms apresentaram aumento proporcionalmente maior que Ra (Figura 1, p.20). Portanto, os resultados sugerem que no padrão tipo 1 de desmineralização, de 15s para 30s, ocorreu aumento na diferença vertical entre os picos e vales, correspondentes respectivamente à periferia e ao centro do prisma. Isto se traduziu numa melhor definição no padrão de desmineralização, com aprofundamento no centro do prisma. Os cortes de perfil permitem visualizar que a diferença em altura entre o pico e o vale aumentou, porém, a linha do perfil correspondente à largura das paredes dos prismas se manteve regular (Figura 3, p.22).

O resultado deste estudo sugere que a diferença de rugosidade encontrada entre T15 e T30 pode não representar alterações na superfície que favorecem a formação de *micro-tags* (zona híbrida). A melhor definição do padrão de desmineralização em T30, é determinada pelo aumento das grandes diferenças verticais expressas pelo Rz, e é favorável à formação dos *macro-tags*. Porém a

contribuição destes para a efetividade da adesão ainda é questionável (Van Meerbeek et al. 2003).

A adesão é usualmente mensurada pelos testes mecânicos de cisalhamento que buscam avaliar o impacto das variáveis sobre o desempenho clínico. Barkmeier et al. 1987, Wang e Lu 1991, Triolo et al. 1993, concluíram que o tempo de 15 segundos de condicionamento ácido proporciona resistência adesiva semelhante ao de 30 segundos. No presente estudo, o aumento do tempo de condicionamento provocou alterações na superfície do esmalte que provavelmente não promovem melhora significativa na adesão. Portanto, o aumento do tempo de condicionamento pode levar somente a maiores perdas de material dentário (Wang et al. 2005; Watari 2005), sem um aumento real na retenção do material resinoso ao esmalte.

Conclui-se que a rugosidade aumenta com o tempo de condicionamento. Este aumento é maior de 0 para 15s quando comparado com de 15s para 30s. As alterações da superfície do esmalte condicionado de 15s para 30s ocorrem principalmente pelo aumento da altura (Rz) e aprofundamento do centro do prisma.

Estudos adicionais devem ser realizados para avaliar a real contribuição da rugosidade nos valores de adesão.

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TABELAS

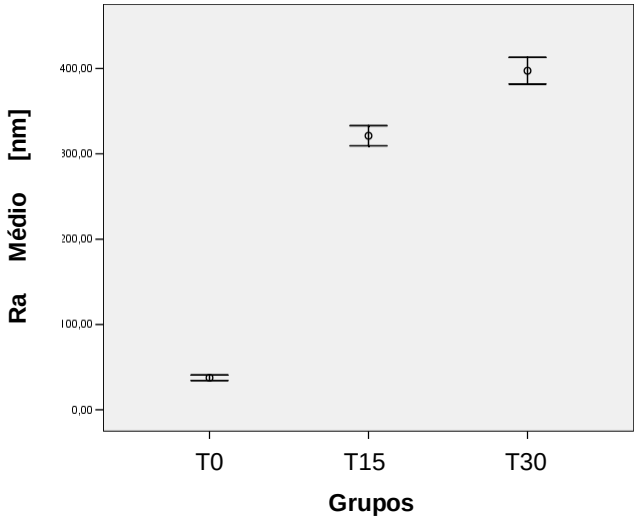
Tabela 1. ESTATÍSTICA DESCRITIVA DAS VARIÁVEIS DE RUGOSIDADE DO ESMALTE Ra, Rz E Rms (µm) PARA OS GRUPOS CONTROLE (T0) E CONDICIONADO COM ÁCIDO FOSFÓRICO 35% POR 15S (T15) E 30S (T30)

Variável	Grupos	n	Média	Desvio Padrão
Ra	T0	30	0,037	0,009
	T15	30	0,321	0,031
	T30	30	0,397	0,042
Rz	T0	30	0,309	0,108
	T15	30	1,695	0,232
	T30	30	2,212	0,337
Rms	T0	30	0,049	0,011
	T15	30	0,389	0,036
	T30	30	0,481	0,046

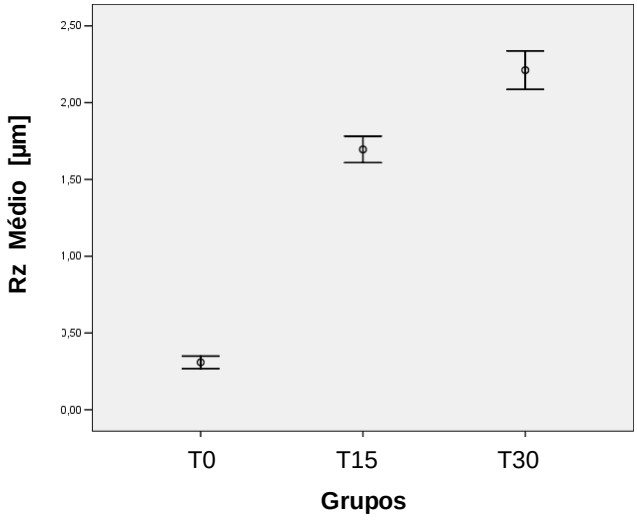
FIGURAS

Figura 1.

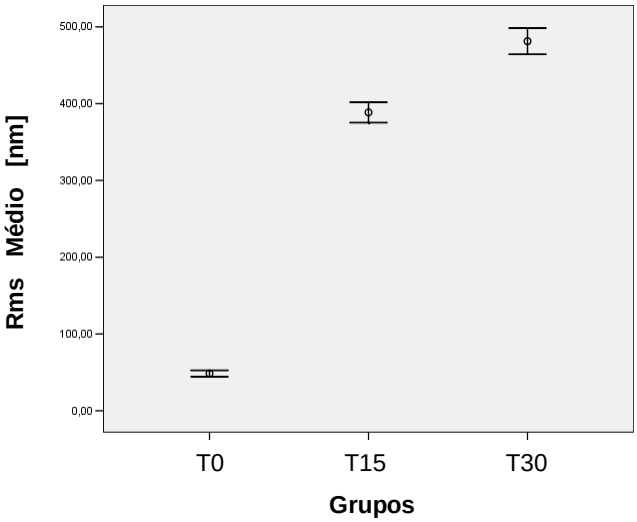
(A)



(B)



(C)



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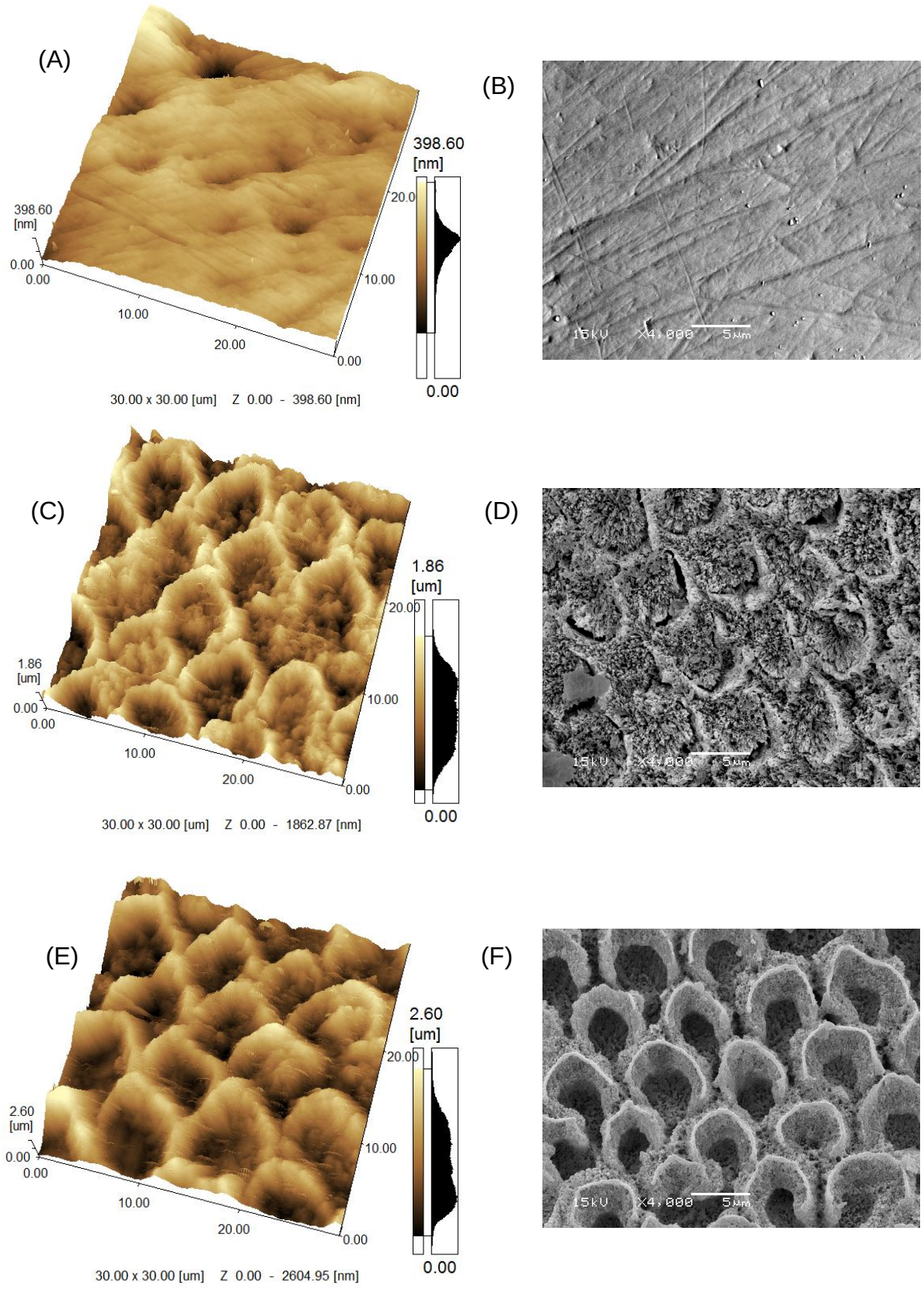
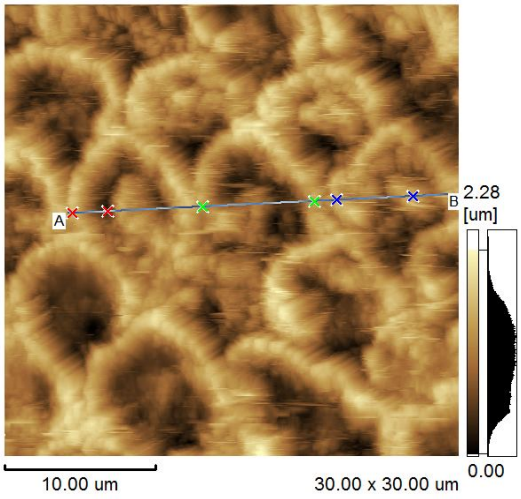
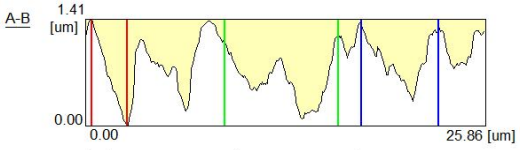

(A)
398.60
[nm]
0.00
0.00
10.00
20.00
0.00
398.60
[nm]
20.00
10.00
0.00
30.00 x 30.00 [um] Z 0.00 - 398.60 [nm]
(B)
15kV X4,000 5μm
(C)
1.86
[um]
0.00
0.00
10.00
20.00
0.00
1.86
[um]
20.00
10.00
0.00
30.00 x 30.00 [um] Z 0.00 - 1862.87 [nm]
(D)
15kV X4,000 5μm
(E)
2.60
[um]
0.00
0.00
10.00
20.00
0.00
2.60
[um]
20.00
10.00
0.00
30.00 x 30.00 [um] Z 0.00 - 2604.95 [nm]
(F)
15kV X4,000 5μm

Figura 3.

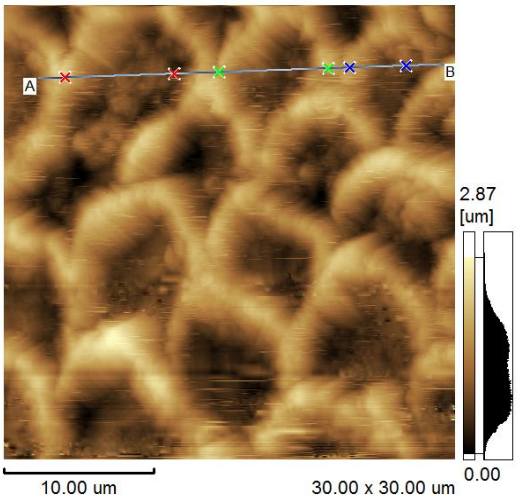
(A)

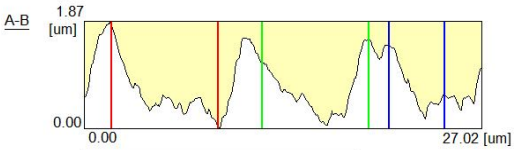




	Largura [um]	Altura [um]
—	2.30	1.41
—	7.37	0.09
—	5.01	0.06

(B)





	Largura [um]	Altura [um]
—	7.28	1.75
—	7.28	0.41
—	3.79	0.86

LEGENDAS

Figura 1. Diferenças dos valores médios das variáveis (A) Ra, (B) Rz e (C) Rms entre os grupos T0, T15 e T30.

Figura 2. (A,C,E) Imagens tridimensionais de 30X30µm (MFA). (B,D,F) Fotomicrografias na magnificação de 4000X (MEV). O esmalte dentário não condicionado em T0, (A,B) apresentou uma superfície regular e eventuais riscos, devido à profilaxia com pedra-pomes. O esmalte condicionado com ácido fosfórico 35% em T30 (E, F) revelou que o padrão de desmineralização tipo 1 é mais definido, e aprofundado no centro do prisma, do que em T15 (C, D). E, a estrutura de “favos-de-mel” ou “buraco de fechadura” pode ser observada no esmalte condicionado (C,D,E,F).

Figura 3. Imagens bidimensionais de 30X30µm (MFA) do esmalte dentário condicionado com ácido fosfórico 35%. Na comparação entre as linhas de perfil A-B em T15(A) e T30(B) a altura do pico ao vale foi maior para T30 (vermelho), a largura do prisma e as paredes dos prismas não tiveram alterações significativas entre T15 e T30 (verde), e a altura do pico ao centro do prisma foi maior para T30 (azul) em que ocorreu maior desmineralização e aprofundamento do centro do prisma.

2. ARTIGO EM INGLÊS

TITLE PAGE

Dental Enamel Roughness with Different Acid Etching Times: An Atomic Force Microscopy Study

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ABSTRACT

Since the introduction of acid etching to aid adhesion to enamel, little has been done to examine the surface topography of etched enamel, although many studies have examined dental materials to improve bond strength. An important characteristic of human dental enamel not yet fully studied is its surface roughness at the microscopic level. The purpose of this study was to quantitatively and qualitatively evaluate the surface topography of acid-etched enamel with different etching times. Ninety six human maxillary bicuspids, after pumice prophylaxis, were randomly distributed in 3 groups (n=32): T0 (control), pumiced; T15, 35% phosphoric acid-etched enamel for 15s; T30, 35% phosphoric acid-etched enamel for 30s. Three-dimensional images of the topography of the enamel were obtained with atomic force microscopy (AFM), and were also quantitatively analyzed to obtain Ra (mean arithmetic roughness), Rz (mean distance between five peak maximums and five valley minimums), and Rms (root mean square roughness). Additionally, photomicrographs were obtained by scanning electronic microscopy (SEM) to illustrate the results. ANOVA and Games-Howell multiple comparisons test were used to analyze the data. Roughness variables Ra, Rz, and Rms showed statistically significant differences in all groups (p<0.000), with values increasing with etching time. This increase was greater from 0 to 15 seconds when compared to 15 to 30 seconds. Enamel surface alterations in T15 to T30 occur mainly due to the increase in height (Rz) and deepening of the central region of the prism. In conclusion, enamel roughness progressively increases with acid etching time up to 30 seconds.

Key-words: Topography, Dental Enamel, Acid Etching, Atomic Force Microscopy, Electron Scanning Microscopy.

INTRODUCTION

With the advent of acid etching on dental enamel in the 1950s (Buonocore 1955), adhesion was introduced into dentistry. Acid etching causes selective demineralization that increases the free surface energy, enamel porosity, and surface area (Beech and Jalaly 1980). Adhesion to enamel is dependent on the capacity of resins to penetrate between rods and crystals (Shinchi et al. 2000), resulting in micromechanical retention. Infiltrated resin encapsulates individually hydroxyapatite crystals creating micro-tags (Van Meerbeek et al. 2003) and constitutes the hybrid layer which promotes a nanoretention mechanism between the dental structure and resinous material (Hannig et al. 2002; Nakabayashi and Pashley 1998). Micro-tags probably contribute more to adhesion efficacy than macro-tags, which fill the space surrounding the enamel prisms (Van Meerbeek et al. 2003).

The retention characteristics of etched enamel surfaces depend on chemical composition, acid type and concentration, and etching time (Carstensen 1992; Legler et al. 1989; Powers et al. 1997). Studies have demonstrated that varying etch time from 15 to 90 seconds, with 35-37% phosphoric acid, does not present a significant impact on shear bond strength of orthodontic brackets. (Barkmeier et al. 1987; Triolo et al. 1993; Wang and Lu 1991).

Although many studies have been devoted to the characterization of acid-etched enamel surfaces (Poole and Johnson 1967; Marshall et al. 1975 Silverstone et al. 1975; Galil and Wright 1979a,b; Oliver 1987; Gardner and Hobson 2001; Hobson et al. 2001, 2002; Hobson and McCabe, 2002), the majority of these studies used electron scanning

microscopy (SEM) and, therefore, provided only qualitative data on dental enamel topography. The etch patterns observed with SEM by Galil and Wright (1979) were classified into five types: 1) preferential dissolution of the prism cores, resulting in a honeycomb-like appearance; 2) preferential dissolution of the prism peripheries, giving a cobblestone-like appearance; 3) a mixture of type 1 and type 2 patterns; 4) pitted enamel surfaces as well as structures that look like unfinished maps or networks; and 5) flat, smooth surfaces.

Traditionally, surface roughness is expressed by a measurement that represents an averaged macroscopic measurement of the overall surface topography. Microscopic surface details may be neglected due to instrument limitations. Atomic force microcopy (AFM), with high lateral and vertical resolutions, allows the exploration of this roughness at a low scale that is not significantly influenced by macroscopic components such as surface waviness, which are less relevant for studying processes involving microscopic particles (Mendez-Vilas et al. 2007).

The effect of surface roughness on adhesion is not completely understood (Gardner and Hobson 2001). However, Eick et al. (1972) asserted that if a surface is roughened, producing more surface area, and, if intimate contact between the adhesive and the adherend is established, the actual adhesive bonding will be stronger because of the increase in surface area.

Since the introduction of acid etching to aid adhesion to enamel, there has been much research into dental materials to improve bond strength, but little into the surface topography of etched enamel (Hobson et al. 2002b). An important characteristic of human dental enamel not yet studied in detail is its surface roughness at the

microscopic level (Casas et al. 2008). Therefore, the aim of this study was to compare and qualitatively and quantitatively analyze the enamel roughness alterations that occur with acid etching for 15 and 30 seconds.

MATERIALS AND METHODS

Enamel Specimen Preparation

In total, 96 intact human maxillary bicuspids, which had been extracted for orthodontic reasons, were obtained from the Tooth Bank of the Pontifical Catholic University of Paraná. The teeth were disinfected in a 0.5% chloramine-T solution for seven days, and then stored in distilled water at 4ºC, which was changed every 7 days, in accordance with ISO/TS 11405. All procedures were approved by the Ethics Committee of Research of the Pontifical Catholic University of Paraná.

The specimens were obtained by cutting the buccal face of all maxillary bicuspids with a flexible perforated diamond disc 7015 (KG Sorensen, Barueri, SP, Brazil), maintaining the middle third of the buccal face parallel to the cutting plane. Pumice prophylaxis slurry was applied for 10 seconds, and then the specimens were rinsed with distilled water for 20 seconds and air dried for 20 seconds. Teeth were randomly distributed into 3 groups (n=32) and treated as follows: T0 (control), pumiced enamel; T15, 35% phosphoric acid (3M Dental Products Division, St. Paul, MN) was applied for 15 seconds, rinsed with distilled water for 20 seconds, and air dried for 20 seconds; T30, 35% phosphoric acid was applied for 30 seconds, rinsed with distilled water for 20 seconds, and air dried for 20 seconds. Thirty specimens from each group were analyzed by AFM, and two were analyzed by SEM.

Atomic Force Microscopy (AFM)

All images were obtained with a Shimadzu SPM-9500J3 AFM (Shimadzu Co., Tokyo, Japan) using a silicon nitrate pyramidal contact tip (Olympus, Tokyo, Japan) in

constant force mode, scanned area sized 30μmx30μm, maximum vertical amplitude (Z) of 5μm, frequency of 1Hz, 512 lines taken per image, and operating point of 2V.

Three images per specimen of the middle third of the buccal face were selected for surface analysis. The majority of the images presented the type 1 etch pattern (Galil and Wright 1979). Thus, only this pattern was used to prevent the macro-geometric topography of different etching patterns from causing an incorrect evaluation of the micro-roughness, the main objective of this study. The images were flattened with the software *SPM Manager v2.11* (Shimadzu), and then the surface analysis was applied to obtain the following data: Ra (mean arithmetic roughness), Rz (mean distance between five peak maximums and five valley minimums), and Rms (root mean square roughness), and profile analysis (to illustrate the results).

Scanning Electron Microscopy (SEM)

A total of 6 samples were coated with 15 nm gold and stored in a desiccator for 48 hours. Photomicrographs with x4000 magnification were taken with a Jeol JSM 6360-LV SEM (Tokyo, Japan), operated with 15 kV to illustrate the results.

Statistical Analysis

The mean of the variables Ra, Rz, and Rms were obtained from 270 images, 3 of each of the 90 specimens.

The variance homogeneity test of Levene indicated that the groups presented heterogeneous variance for all variables. ANOVA and the Games-Howell multiple comparisons test were used to illustrate the differences among the variables mean

values of the different groups. Pearson's correlation test was used to correlate the variables.

RESULTS

AFM

Table 1 describes the variables Ra, Rz, and Rms for the three groups. All variables presented statistically significant differences among groups (p<0.0000). The roughness increased, non-linearly, with an increase in etching time (Figure 1). Ra, Rz, and Rms measurements presented high correlations with each other (R>0.959, p<0.0000).

Three-dimensional images of the type 1 etch pattern of acid-etched enamel for 30 seconds (Figure 2e) presented greater mineral removal of the prism core region than the enamel etched for 15 seconds (Figure 2c).

SEM

Pumiced enamel presented a regular surface with eventual wear (Figure 2b), and 35% phosphoric acid-etched enamel for 30 seconds (Figure 2d) presented the type 1 etch pattern, which was better defined than for 15 seconds (Figure 2f).

DISCUSSION

Roughness is defined as a complex role of irregularities, or, little projections and indentations that characterize a surface and influence wetting, the quality of adhesion, and brightness. Although micro-mechanical roughness is necessary to obtain efficient adhesion to dental enamel (Gwinnett and Matsui 1967; Buonocore et al. 1968), the precise characteristics of etched enamel and the scale at which adhesion occurs are not known.

An important characteristic of human dental enamel not fully studied is its surface roughness at the microscopic level (Casas et al. 2008). AFM allows us to further understand the microstructure of tissues and demineralization patterns , as well as the physical-chemical processes at the enamel surface, both qualitatively and quantitatively (Jandt et al. 2000).

Demineralization patterns, when statically analyzed, can be differentiated and classified (Galil and Wright 1979). Dissolution does not occur equally on all enamel surface with etching time due to apatite crystallite orientation, initiating at the core/wall interfaces of rods and developing anisotropically along the c-*axis* (Wang et al., 2005). In the present study, enamel etched with 35% phosphoric acid for 30 seconds (Figures 2e,f) revealed a type 1 etching pattern that was better defined and deeper in prism cores than for 15 seconds (Figures 2c,d). In agreement with Gardner and Hobson (2001), the type 1 and 2 patterns (made with 37% phosphoric acid) at 30 seconds were better defined than at 15 seconds.

In agreement with a study by Carstensen (1992), the relationship between etch pattern and bond strength indicated that the type 1 and 2 patterns promote maximum adhesion. Additionally, the longevity of bonding is influenced by etch pattern (Hobson et al. 2002a) However, Nakabayashi and Pashley (1998) suggested that resin-enamel bond strength is the result of the cumulative cross-sectional area of the resin tags that infiltrate the etched enamel surface. Therefore, exposure of enamel crystallites is more important than well-defined etch patterns (Hobson et al. 2002a). Orellana et al. (2008) suggested that enamel porosity is more important than defined etch patterns by using the BET method (gas adsorption), in which they did not observe a correlation between the specific surface area and the different etch patterns.

In the present study, the increase in roughness was related to acid etching time, although this progression was not linear; it increased less with respect to time. There was a greater increase in all roughness variables from T0 to T15, than from T15 to T30. However, the quantity of removed mineral from the demineralization process was linear with etching time. (Wang et al. 2005). Watari (2005) pointed out the same behavior to depth using 2% phosphoric acid to etch enamel for up to 3 minutes, and mean roughness (Ra) presented lower increases with etching time.

Roughness measurements Ra and Rms represented, respectively, the mean arithmetic and quadratic deviations of roughness related to the mean profile of the surface. The measurement Rms, therefore, is more sensitive to extreme values (Gadelmawla et al. 2002), which are represented by the roughness measurement Rz. Despite the measurement Ra being used as the sole indicator of surface texture, it cannot differentiate the depth of irregularities (Eliades et al. 2004), and thus peaks and

valleys are registered in an identical manner (Whitehead et al. 1996). Among the groups, the measurements Rz and Rms increased proportionally more than Ra (Figure 1). Therefore, the results suggest that in the type 1 etch pattern, from 15 to 30 seconds, the vertical difference between maximum peaks and valleys increased, corresponding respectively to the periphery and to the core of the prism. Thus, the etch pattern presented a better definition, with deepening in the core of the prisms. The profile analysis allowed us to visualize that the differences in height between peaks and valleys increased, while the prism wall widths were regularly maintained (Figure 3).

The results of this study suggest that the differences in roughness between T15 and T30 may not represent surface alterations that aid the formation of micro-tags, thus enhancing hybrid layer formation. The better definition of etch pattern at T30, is determined by the increase of the vertical differences expressed by Rz, and is favorable to form macro-tags. However, the contribution of these to adhesion effectiveness is still questionable (Van Meerbeek et al. 2003).

Adhesion is usually measured by shear bond strength tests that evaluate the impact of variables on clinical performance. Barkmeier et al. (1987), Wang and Lu (1991), and Triolo et al. (1993) concluded that 15 seconds of acid etching provides bond strength similar to 30 seconds. In the present study, the increase in etching time caused alterations on enamel surface that probably do not promote significant enhancement in adhesion. Therefore, the increase in etching time may lead only to greater loss of dental material (Wang et al. 2005; Watari 2005), without an increase in the retention of resinous material to the enamel.

In conclusion, enamel roughness increased with increasing etching times. This increase is greater from 0 to 15 seconds when compared to 15 to 30 seconds. Enamel surface alterations from T15 to T30 occurred mainly due to an increase in height (Rz) and deepening of the central region of the prism. Additional studies should be conducted to evaluate the contribution of dental enamel roughness to adhesion values.

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TABLES

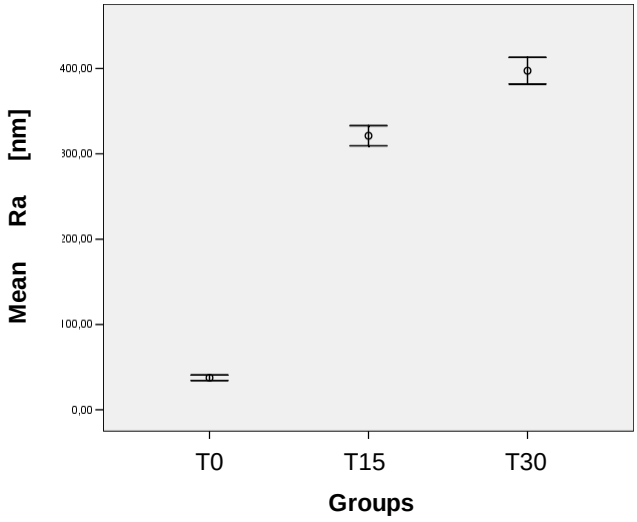
Table 1. Descriptive statistics of roughness variables Ra, Rz, and Rms (μm) for the etching time groups T0, T15, and T30 (sec)

Variable	Groups	n	Mean	Standard Deviation
Ra	T0	30	0.037	0.009
	T15	30	0.321	0.031
	T30	30	0.397	0.042
Rz	T0	30	0.309	0.108
	T15	30	1.695	0.232
	T30	30	2.212	0.337
Rms	T0	30	0.049	0.011
	T15	30	0.389	0.036
	T30	30	0.481	0.046

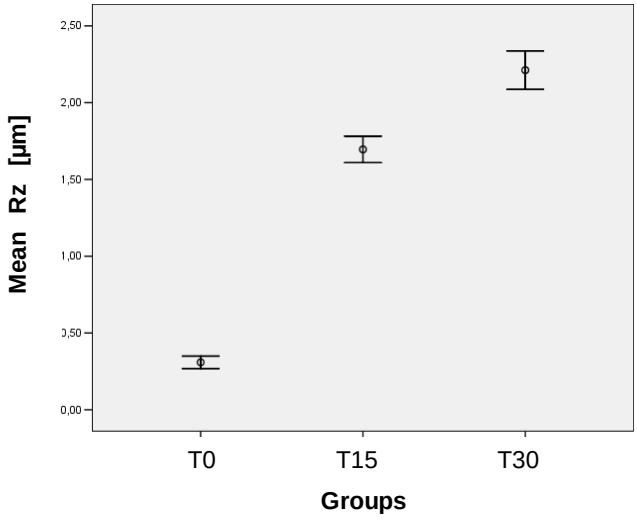
FIGURES

Figure 1.

(A)



(B)



(C)

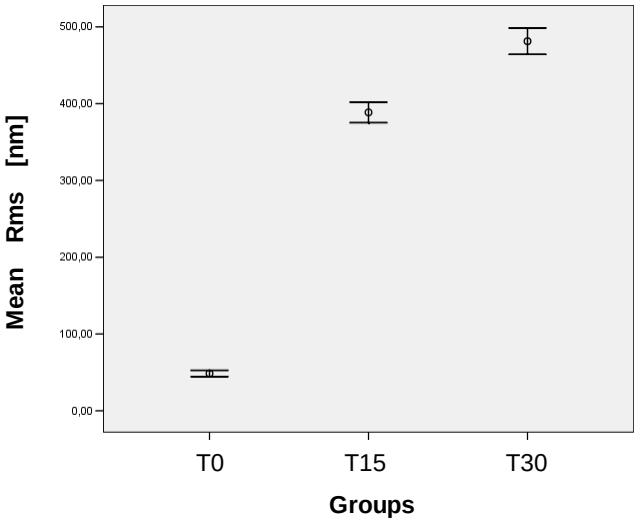


Figure 2.

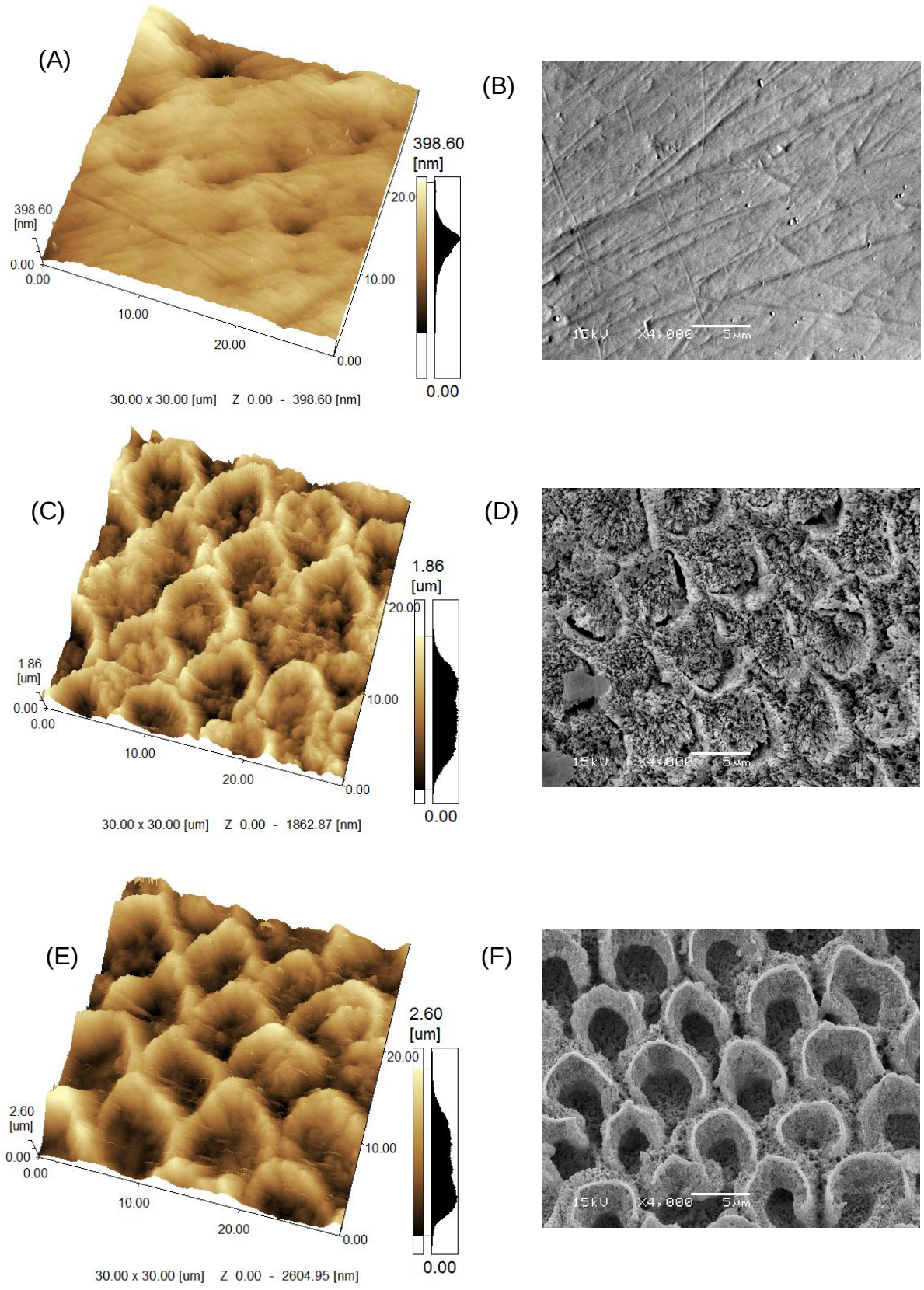
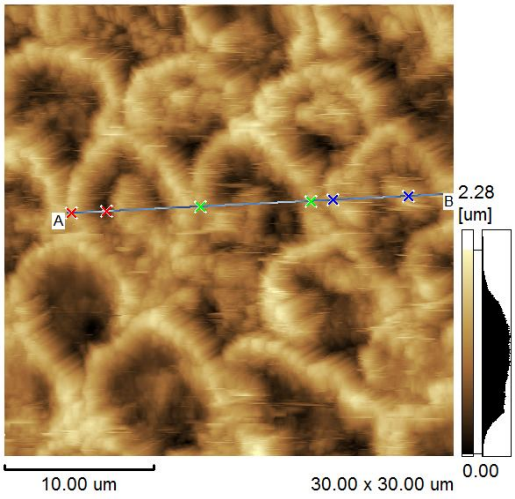
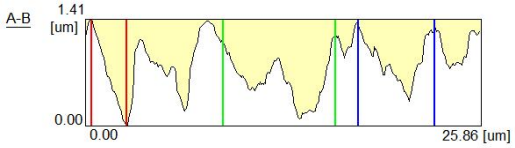

(A)
398.60
[nm]
0.00
0.00
10.00
20.00
0.00
398.60
[nm]
20.0
10.00
0.00
30.00 x 30.00 [um] Z 0.00 - 398.60 [nm]
(B)
15kV X4,000 5μm
(C)
1.86
[um]
0.00
0.00
10.00
20.00
0.00
1.86
[um]
20.00
10.00
0.00
30.00 x 30.00 [um] Z 0.00 - 1862.87 [nm]
(D)
15kV X4,000 5μm
(E)
2.60
[um]
0.00
0.00
10.00
20.00
0.00
2.60
[um]
20.00
10.00
0.00
30.00 x 30.00 [um] Z 0.00 - 2604.95 [nm]
(F)
15kV X4,000 5μm

Figure 3.

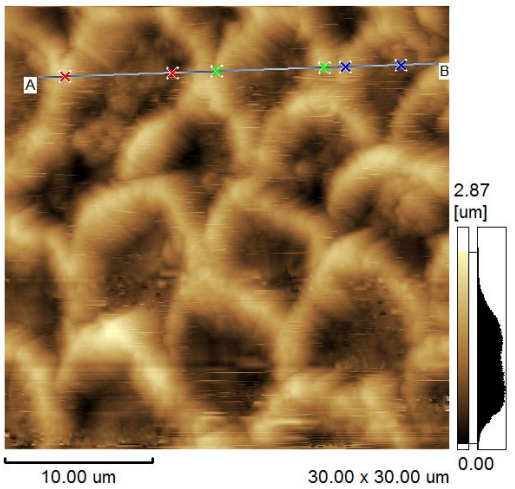
(A)

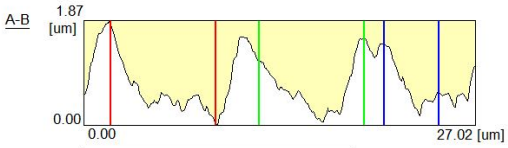




	Width [um]	Height [um]
—	2.30	1.41
—	7.37	0.09
—	5.01	0.06

(B)





	Width [um]	Height [um]
—	7.28	1.75
—	7.28	0.41
—	3.79	0.86

FIGURE LEGENDS

Figure 1. Mean value of the different variables (A) Ra, (B) Rz , and (C) Rms between groups T0, T15, and T30.

Figure 2. (A,C,E) Three-dimensional images, sized 30 μm X30μm (AFM). (B,D,F) Photomicrographs in X4000 magnifications (SEM). (A,B) Dental enamel that had been pumiced presented a regular surface with eventual wear, due to prophylaxis with the pumice slurry. (E,F) The enamel that was acid-etched for 30 seconds with 35% phosphoric acid revealed a better defined type 1 etch pattern, and deepened prism cores than (C, D) for 15 seconds. Thus, the honeycomb structure could be observed in the etched enamel (C, D, E, F).

Figure 3. AFM profile analysis of 35% phosphoric acid-etched dental enamel. Profile lines A-B comparison between T15 (A) and T30 (B): peak to valley height was higher for T30 (red), prism width and walls were not altered significantly between T15 and T30 (green), and the prism peak to core height was higher at T30 (blue), which presented greater demineralization and prism core depth.

3.ANEXOS

ANEXO I – PARECER DO COMITÊ DE ÉTICA EM PESQUISA DA PUCPR

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ
Núcleo de Bioética
Comitê de Ética em Pesquisa
Ciência com Consciência

PARECER CONSUBSTANCIADO DE PROTOCOLO DE PESQUISA

Parecer Nº **0002075/08** Protocolo CEP Nº **2638**

Titulo do projeto **Topografia do esmalte dentário com diferentes tempos de condicionamento ácido: estudo em microscopia eletrônica de varredura e microscopia de força atômica** Grupo **III** Versão **1**

Protocolo CONEP **0406.0.084.000-08** Pesquisador responsável **Orlando Motohiro Tanaka**

Instituição

Objetivos

Comparar quantitativa e qualitativamente a topografia do esmalte dentário condicionado com ácido fosfórico 37% nos tempos 0, 15 e 30 segundos.

Comentários

O presente estudo tem como objetivo a obtenção de dados quantitativos com resolução nanométrica, filtrando-se as ondulações macrométricas, assim como da imagem tridimensional da topografia do esmalte, possibilitando comparar o aumento da rugosidade do esmalte com diferentes tempos de condicionamento com ácido fosfórico 37% e evidenciar as alterações ocorridas na superfície do dente.

Considerações

O projeto apresenta metodologia e delineamentos adequados.

Termo de consentimento livre e esclarecido

Não se aplica no presente trabalho.

Conclusões

O projeto de trabalho atende as recomendações da Res. 196/96, do CNS.

Devido ao exposto, o Comitê de Ética em Pesquisa da PUCPR, de acordo com as exigências das Resoluções Nacionais 196/96 e demais relacionadas a pesquisas envolvendo seres humanos, em reunião realizada no dia: **01/10/2008**, manifesta-se por considerar o projeto **Aprovado**.

Comitê de Ética em Pesquisa em Seres Humanos - CEP PUCPR

Parecer Nº **0002075/08** Protocolo CEP Nº **2638**

Titulo do projeto **Topografia do esmalte dentário com diferentes tempos de condicionamento ácido: estudo em microscopia eletrônica de varredura e microscopia de força atômica** Grupo **III** Versão **1**

Protocolo CONEP **0406.0.084.000-08** Pesquisador responsável **Orlando Motohiro Tanaka**

Instituição

Situação Aprovado

Lembramos aos senhores pesquisadores que, no cumprimento da Resolução 196/96, o Comitê de Ética em Pesquisa (CEP) deverá receber relatórios anuais sobre o andamento do estudo, bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de eventos adversos, para conhecimento deste Comitê. Salientamos ainda, a necessidade de relatório completo ao final do estudo.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP-PUCPR de forma clara e sucinta, identificando a parte do protocolo a ser modificado e as suas justificativas.

Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciá-la antes de receber a autorização formal para a sua realização. O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do pesquisador responsável, podendo ser requerido por este CEP em qualquer tempo.

Curitiba, 01 de Outubro de 2008.

Prof. Dr. Sergio Surugi de Siqueira
Coordenador do Comitê de Ética em Pesquisa
PUCPR

Comitê de Ética em Pesquisa em Seres Humanos - CEP PUCPR

ANEXO II - ANÁLISE ESTATÍSTICA

TABELA 1. ESTATÍSTICA DESCRITIVA DAS VARIÁVEIS Ra, Rz E Rms (µm) PARA OS GRUPOS T0, T15 E T30, PUCPR - 2008

Variável	Grupos	n	Média	Mediana	Desvio Padrão	Erro Padrão
Ra	T0	30	0,037	0,036	0,009	0,002
	T15	30	0,321	0,316	0,031	0,006
	T30	30	0,397	0,406	0,042	0,008
Rz	T0	30	0,309	0,287	0,108	0,020
	T15	30	1,695	1,712	0,232	0,042
	T30	30	2,212	2,204	0,337	0,062
Rms	T0	30	0,049	0,047	0,011	0,002
	T15	30	0,389	0,385	0,036	0,007
	T30	30	0,481	0,489	0,046	0,008

Fonte: dados da pesquisa

TABELA 2. TESTE DE HOMOGEINEDADE DE VARIÂNCIAS DE LEVENE PARA AS VARIÁVEIS Ra, Rz E Rms, PUCPR - 2008

Variável	Estatística	G.L.1	G.L.2	Valor p
Ra	7,7702	2	87	0,0008*
Rz	5,2991	2	87	0,0067*
Rms	6,5065	2	87	0,0023*

Fonte: dados da pesquisa
NOTA: p < 0,05 indica não homogeinedade de variância

TABELA 3. TESTE DE COMPARAÇÕES MÚLTIPLAS DE GAMES-HOWELL ENTRE OS GRUPOS T0, T15 E T30 PARA A VARIÁVEL DEPENDENTE Ra, PUCPR - 2008

(I) Grupos	(J) Grupos	Diferença de Médias (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					L.I.	L.S.
T0	T15	-283,6740	5,9541	0,0000*	-298,2784	-269,0697
	T30	-359,8696	7,7741	0,0000*	-378,9896	-340,7495
T15	T0	283,6740	5,9541	0,0000*	269,0697	298,2784
	T30	-76,1955	9,5387	0,0000*	-99,1845	-53,2066
T30	T0	359,8696	7,7741	0,0000*	340,7495	378,9896
	T15	76,1955	9,5387	0,0000*	53,2066	99,1845

Fonte: dados da pesquisa
NOTA: * indica diferença estatisticamente significante (p<0,05)

TABELA 4. TESTE DE COMPARAÇÕES MÚLTIPLAS DE GAMES-HOWELL ENTRE OS GRUPOS T0, T15 E T30 PARA A VARIÁVEL DEPENDENTE Rz, PUCPR - 2008

(I) Grupos	(J) Grupos	Diferença de Médias (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					L.I.	L.S.
T0	T15	-1,3864	0,0466	0,0000*	-1,4998	-1,2730
	T30	-1,9026	0,0647	0,0000*	-2,0609	-1,7444
T15	T0	1,3864	0,0466	0,0000*	1,2730	1,4998
	T30	-0,5163	0,0747	0,0000*	-0,6966	-0,3359
T30	T0	1,9026	0,0647	0,0000*	1,7444	2,0609
	T15	0,5163	0,0747	0,0000*	0,3359	0,6966

Fonte: dados da pesquisa
NOTA: * indica diferença estatisticamente significante (p<0,05)

TABELA 5. TESTE DE COMPARAÇÕES MÚLTIPLAS DE GAMES-HOWELL ENTRE OS GRUPOS T0, T15 E T30 PARA A VARIÁVEL DEPENDENTE Rms, PUCPR - 2008

(I) Grupos	(J) Grupos	Diferença de Médias (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					L.I.	L.S.
T0	T15	-339,9381	6,8364	0,0000*	-356,6823	-323,1939
	T30	-432,6193	8,6500	0,0000*	-453,8677	-411,3710
T15	T0	339,9381	6,8364	0,0000*	323,1939	356,6823
	T30	-92,6813	10,6579	0,0000*	-118,3579	-67,0046
T30	T0	432,6193	8,6500	0,0000*	411,3710	453,8677
	T15	92,6813	10,6579	0,0000*	67,0046	118,3579

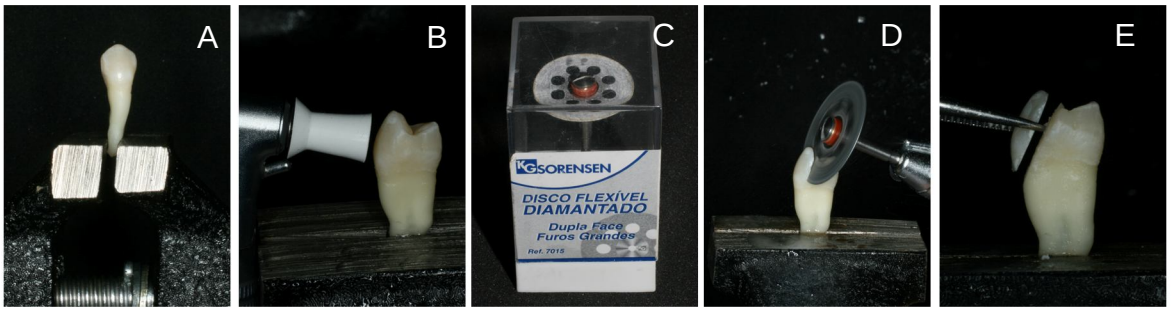
Fonte: dados da pesquisa
NOTA: * indica diferença estatisticamente significante (p<0,05)

TABELA 6. TESTE DE CORRELAÇÃO DE PEARSON ENTRE AS VARIÁVEIS Ra, Rz e Rms, PUCPR - 2008

	n	R	Valor p
Ra & Rz	90	0.9591	0.0000*
Ra & Rms	90	0.9998	0.0000*
Rz & Rms	90	0.9616	0.0000*

Fonte: dados da pesquisa
NOTA: * indica diferença estatisticamente significante (p<0,05)

ANEXO III – METODOLOGIA ESTENDIDA



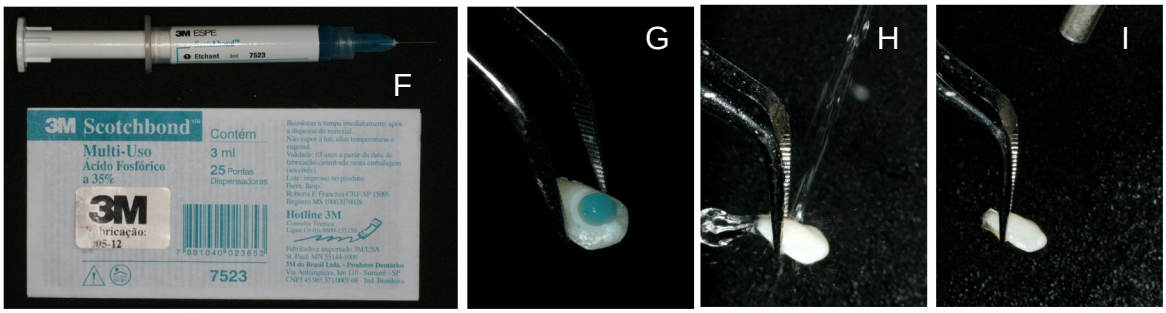
A – Pré-molar superior fixado na morsa para preparo do corpo de prova

B – Profilaxia com pedra-pomes do esmalte dentário da face vestibular

C – Disco flexível diamantado dupla face com furos grandes (KG SORENSEN 7015)

D – Secção da face vestibular mantendo o terço médio da face vestibular paralelo ao plano de corte

E – Corpo de prova seccionado

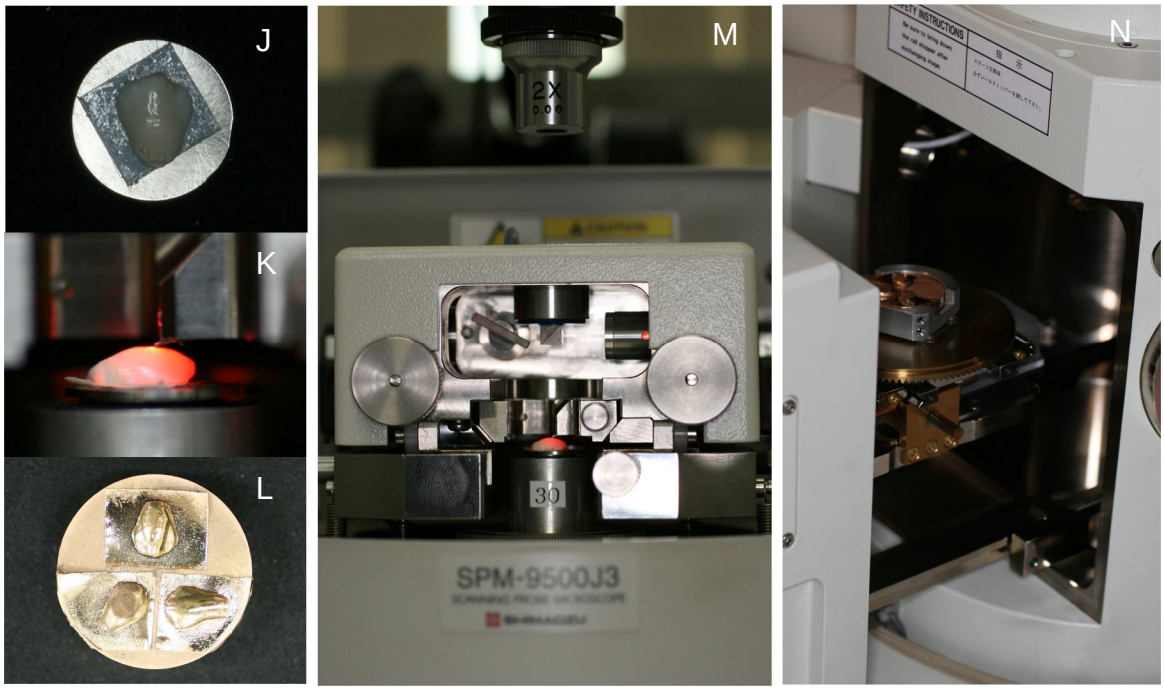


F – Ácido Fosfórico 35% (3M Scotchbond)

G – Condicionamento ácido do esmalte dentário

H – Lavagem por 20 segundos

I – Secagem por 20 segundos



J – Vista superior do corpo de prova fixado com fita dupla-face (3M) sobre o porta amostra do Microscópio de Força Atômica (MFA)

K, M – Corpo de prova posicionado no MFA (Shimadu SPM-9500J3)

L – Vista superior dos corpos de prova fixados com fita dupla-face de cobre (3M) sobre os *stubs* de alumínio, metalizados com ouro, do Microscópio Eletrônico de Varredura (MEV)

N – Corpo de prova posicionado no MEV (JEOL JSM 6360-LV)

ANEXO IV - Normas para Publicação – *JOURNAL OF DENTAL RESEARCH*

Instructions to Contributors

GENERAL POLICY

Submitted manuscripts must be written clearly and concisely in English and represent unpublished original research that is not being considered for publication elsewhere. The *Journal* seeks to publish (i) concise definitive reports of wide interest to the research community and (ii) topical, concise reviews of the state of the art. Concise definitive reports of findings of unusual significance can be reviewed as (iii) Rapid Communications. Criteria for the acceptance of Rapid Communications will be more stringent than those for regular papers. Authors should request review for Rapid Communications in the cover letter to the Editor. The *Journal* also welcomes (iv) essays that explore seminal events and creative advances in the development of dental research for publication in the "Discovery!" column.

Reports of observations and the development of new methods or techniques may be considered for publication if they are of broad and fundamental interest, but the focus of the Journal is on definitive reports demonstrating cause-and-effect relationships. Submission of case reports is generally discouraged.

Letters to the Editor must include evidence to support a position about the scientific or editorial content of the *Journal* and are limited to 250 words. Guest Editorials describe a clear and substantiated position on issues of interest to the community and are also encouraged. These are limited to 1000 words. As appropriate, a brief response to Letters or Guest Editorials will be solicited for concurrent publication. Conference Reports will be considered for publication only if they are topical and brief, highlighting important new data or findings. Final approval for publication rests with the Editor.

Authors considering submitting a Conference Report should first consult the Editor regarding the suitability of the report. Such reports should: (a) be of broad and international interest to the readership of the *JDR*; (b)include the specific aims of the meeting, the pertinent information provided by the speakers (i.e., summary of a few sentences from each talk), then the specific conclusions; and (c) meet the same word limits and figure/table numbers as a regular research report. An abstract is not required. Priority for publication would depend on our publishing schedule for regular research reports, which generally carry higher priority.

Submitted manuscripts must be accompanied by a cover letter with the signatures of all authors certifying that the research is (i) original, (ii) not presently under consideration for publication elsewhere, (iii) free of conflict of interest (e.g., edited by the funding agency or organization), and (iv) conducted by the highest principles of human subject and animal welfare. In the cover letter, contributors should nominate four expert, independent scientific referees and include their names, mailing addresses, telephone/FAX numbers, e-mail addresses, and area of expertise. It is highly encouraged that referees be nominated to avoid delays in the review process. Nominated scientific referees may not be colleagues at the contributors' institutions or present or former collaborators. The receipt of all manuscripts will be

acknowledged. The contributors will be notified subsequently by the Editor of (1) acceptance, (2) need for revisions, or (3) rejection.

Also include a list of total words (from Abstract to Acknowledgments), number of words in Abstract, total number of tables and/or figures, and number of references.

All rights in manuscripts shall be transferred to the *Journal of Dental Research* upon submission. Submission of a manuscript shall constitute each author's agreement that the Journal of Dental Research holds all proprietary rights in the manuscript submitted, including all copyrights. On acceptance and before publication of a paper, contributors will be asked to sign a formal transfer of copyright.

For clarity, please use only common abbreviations, which will be widely recognized by the more general reader of the Journal. A list of all abbreviations used should be provided on the manuscript cover page.

RESEARCH REPORTS

(All submissions must adhere to the following criteria.) These will be limited to a maximum of 2500 words (including abstract, introduction, materials & methods, results, discussion, and acknowledgments, but excluding the reference list and figure legends), an abstract containing a maximum of 150 words, a maximum of 35 references, and a combined total of 4 tables/figures. Additional supporting data may be referenced as a supplemental appendix for publication online only. The appendix must be submitted with the manuscript for review. Research Reports will be published in three categories:

- Clinical
- Biomaterials & Bioengineering
- Biological

CONCISE REVIEWS

With the addition of content from the journal *Critical Reviews in Oral Biology & Medicine*, the 'Concise Review' category in *JDR* will be discontinued, and manuscripts for Concise Reviews will no longer be considered.

CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Critical Reviews in Oral Biology & Medicine manuscripts cannot be submitted online. Authors must contact the *Critical Reviews in Oral Biology & Medicine* editor, Dr. Dana Graves, at dgraves@iadr.org for submission instructions.

The manuscript should briefly summarize information that is well known and emphasize recent developments over the last three years. This will help focus the manuscript on cutting edge research. Please stress critical issues and concepts that would add excitement to the article. In addition, visual elements should be added to make the manuscript as "approachable" as possible. The manuscript should follow the following guidelines:

- It is important to include several illustrations or diagrams to enhance clarity. Manuscripts that lack figures or diagrams typically receive a low priority score. The cost of color figures in the print version will be borne by the authors and are approximately $1000 per figure. However, there are

no charges for figures and diagrams printed in black and white. Color figures may be included in the on-line version of JDR with no extra charges.

- Summarize important concepts in tables or flow charts or show critical data in the form of figures. Please note that you will need to obtain permission to reproduce a previously published figure or table.
- The length of the article with references and tables/figures should be approximately 35-40 double spaced, 8 X 11 inch typed pages with a 12 font size. The page limitation is enforced by the Journal. In addition, there should be as few abbreviations as possible. Due to the broad readership abbreviations commonly recognized in one field may not be readily apparent to those in a different field.
- The cover page, abstract (~200 words), text, summary, figure legends and tables should be combined into a single Word document and the Figures sent as a PDF file containing all figures. If the on-line version is to be in color and the printed version in B&W please submit separate PDF files for B&W and color figures. Note that the figures in both PDF files should be identical except that the B&W is in grayscale.
- Examples of recent review articles in JDR may be found at the following links: http://jdr.iadrjournals.org/cgi/content/full/86/9/800 http://jdr.iadrjournals.org/cgi/content/full/85/7/584

DISCOVERY! ESSAYS

Readers are encouraged to submit *Discovery!* essays/articles that they feel will be of interest to the *JDR* readership. Authors are welcome to submit manuscripts for consideration directly to the Discovery! editor, Dr. Marty Taubman, at mtaubman@forsyth.org, or by mail to Dr. Marty Taubman, c/o The Forsyth Institute, 140 The Fenway, Boston, MA 02115, USA. Discovery! manuscripts cannot be submitted online. Discovery! manuscripts will be limited to 2500 words and two tables or figures.

MANUSCRIPTS

Prepare manuscript, tables, legends, and footnotes as double-spaced text (a minimum of 6 mm between lines) formatted for 8-1/2 x 11-inch paper. Top, bottom, and side margins should be one inch, with no indented paragraphs. Figures and tables should not exceed 8-1/2 x 11 inches. Both Macintosh (Framemaker, MacWrite, Word, WordPerfect, Works WP, or WriteNow) and IBM PC (DCA-RFT, FrameMaker, MultiMate, Office Writer, Text, Word for Windows, WordPerfect, WordStar, Works WP, or XYWrite) files will be accepted. Manuscripts should be "clean", i.e., free of tabs and codes. Bold and italic type should appear exactly as they will appear on the printed page. Italicize items that will appear in italics; this will include the genus and species of an organism, g (for gravitational force), Latin words and abbreviations (for example, e.g., i.e., in vitro, in vivo, et al.), and journal names in the References section. Tabs should be used to separate columns within tables. **Do not use elaborate table formatting.**

Use a standard font such as Times New Roman or Arial to avoid misrepresentation of your data on different computers that do not have the unusual or foreign language fonts.

Title and Section Headings

Bold type should be used for the title on page 1. Use upper- and lower-case letters. First-level headings, which include ABSTRACT, INTRODUCTION, MATERIALS & METHODS, RESULTS, DISCUSSION, ACKNOWLEDGMENTS, and REFERENCES should be bold type, all upper-case letters, as shown. Second-level subheads should be bold type, upper- and lower-case. Third-level subheads

should be bold type, upper- and lower-case, with a paragraph indent. Any lower-ranked subheads should be italicized, and in upper- and lower-case. Please type no more than 10 characters per inch. Authors are reminded to include their complete mailing addresses, telephone, FAX, and e-mail addresses, as available. Copies of "in press" and "submitted" manuscripts that will provide essential information for the referees should also be enclosed.

A Few Words About Style

Authors should remember that they are writing to communicate to often-uninformed readers. Here are a few suggestions: Show a clear chronological progression and logic to the development of your ideas throughout the manuscript and within paragraphs and sentences. Speak to the reader in a direct and straightforward voice. Tell the reader your purpose, then provide background, data, and conclusions. You will make your point most effectively by illustrating with a well-chosen example, rather than providing an encyclopedic discourse. In each paragraph and sentence, stick to the subject. For example, if the subject is "biophysical properties", don't write sentences in that paragraph that change the subject to the names of cited contributors. Each sentence should contain only one thought. Write short and simple sentences. Choose the best word so that you say what you mean. To make your information accessible to the widest possible audience, avoid jargon, acronyms, and needless words. Before submission, contributors must review their manuscripts with (i) computer grammar and spelling tools/filters and (ii) a colleague who is expert in English language grammar and syntax. Manuscripts may be returned without review or rejected on the basis of poor English or accepted standards of style. Check to ensure that all listed references, figures, and tables are cited in the text and that all cited references, figures, and tables are presented in appropriate sections. The Editor reserves the right to make changes to improve the clarity of the text. All such changes will be subject to contributors' approval before publication.

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All revisions must be accompanied by a cover letter signed by all authors to the Editor. The letter must (i) detail on a point-by-point basis the contributors' disposition of each of the referees' comments, and (ii) certify that all contributors approve of the revised content and that the manuscript complies with stipulations 'i' through 'iv' in "General Policy". Responses to separate reviewers should be on separate pages. Also include a copy of the revision with all changes highlighted.

RANDOMIZED CLINICAL TRIALS

Effective January 2004, manuscripts reporting a randomized clinical trial should follow the CONSORT guidelines as published in the Annals of Internal Medicine (Ann Int Med 134:657-662, 2001). Click here to download the checklist. This completed checklist file should be uploaded as Supplemental Material.

The *Journal* encourages authors to register their clinical trials in a public trials registry, and we ask authors of manuscripts describing such studies to submit the name of the registry and the study registration number prior to publication. The International Committee of Medical Journal Editors plans to

consider clinical trials for publication only if they have been registered (see *N Engl J Med* 2004;351:1250-1 - http://content.nejm.org/cgi/content/full/351/12/1250). The following registries meet these requirements: http://prsinfo.clinicaltrials.gov and http://controlled-trials.com/isrctn/submission/.

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Prior to submission, the *Journal* asks that novel gene sequences be deposited in a public database (GenBank, http://www.ncbi.nlm.nih.gov/Genbank/submit.html, EMBL, http://www.ebi.ac.uk/embl/Submission/index.html, or DDBJ http://www.ddbj.nig.ac.jp/sub-e.html), and the accession number provided to the JDR. Manuscript submissions including microarray data should (a) include the information recommended by the MIAME http://www.mged.org/Workgroups/MIAME/miame.html guidelines in their submission, and/or (b) identify the submission details for the experimental details to one of the publicly available databases (ArrayExpress http://www.ebi.ac.uk/arrayexpress/ or GEO http://www.ncbi.nlm.nih.gov/geo/).

MANUSCRIPT COMPONENTS

The components of a manuscript should be: 1) title page, 2) abstract, 3) introduction,4) materials and methods, 5) results, 6) discussion, 7) acknowledgments, 8) references, 9) tables, and 10) figure legends. The complete manuscript should be arranged in that order. Number all pages consecutively in the top right-hand corner, including the title page. Label figures clearly. Each figure label must indicate the number corresponding to the citation in the text, an arrow indicating the top, and contributors' abbreviated names.

1) Title Page (page 1)

Type in bold type with only the first letters of the main words capitalized. The title should be brief (not to exceed 60 characters) and illustrative of the key finding. Also type the contributors' initials and last names in upper- and lower-case letters. Use superscript numbers to relate contributors to different departments or institutions, or to indicate a change in address. For the corresponding author who will receive reprint requests, provide the full postal (including ZIP or Postal Code) and e-mail addresses, telephone and FAX numbers, as available. If the corresponding author is not the first author, indicate by a number superscript, and use the phrase "corresponding author", and that individual's e-mail address. The following information must be included on the cover page: 1) a short title (running head) of up to 45 characters; 2) three to five key words; 3) the number of words in the abstract; 4) the number of words in the abstract and the text (excluding tables, figure legends, acknowledgments, and cited references); 5) the number of tables and figures; and 6) the number of cited references. If applicable, include source footnotes on page 1 to indicate prior preliminary publication. For example, state that the work was "Based on a thesis submitted to the graduate faculty, Azimuth University, in partial fulfillment of the requirements for the PhD degree" or that a preliminary report was presented at, or published in... Report all sources of funding in a later section, "Acknowledgments".

2) Abstract (page 2)

A self-standing summary of the text, this section should not exceed one typed page (about 150 words). Concisely describe the (i) background and rationale, (ii) hypothesis or study objective, (iii) design and key methods, (iv) essential results, and (v) conclusions. Avoid abbreviations. The abstract will be re-published separately by information retrieval services.

3) Introduction (page 3)

Briefly and clearly describe the background and rationale for the stated hypothesis to be tested or objective to be studied. Sufficient detail must be provided to permit the interdisciplinary reader to evaluate the results without review of earlier publications. Describe and cite only the most relevant earlier studies; avoid presentation of an exhaustive review of the field. Do not include a summary of the results presented in the manuscript.

4)Materials & Methods

To provide sufficient technical information so that the experiments can be repeated, the (i) experimental or study design, (ii) specific procedures, and (iii) type of statistical analysis must be described clearly and carefully. Use section subheadings in a logical order to title each category or method. Previously published methods should be named (e.g., "ultrasonic treatment" rather than mention of the cited contributors' names) and cited. New methods must be described completely. Present the data that validate the new method. A method used for only part of one experiment may be described briefly in the "Results" section, table footnote, or figure legend. Present descriptive information about large numbers of experimental reagents, microbes, test materials, primer sequences, in tabular form with a brief explanation in the text. Proprietary names and sources of supply of all commercial products must be given in parentheses in the text (name and model of product, company, city, and state or country). Report generic names and terms wherever possible. For protocols involving the use of human subjects or specimens, indicate succinctly that subjects' rights have been protected by an appropriate institutional review board and informed consent was granted. When laboratory animals are used, indicate the level of institutional review and assurance that the protocol ensures humane practices.

5)Results

This section serves only to introduce data in the (i) text, (ii) tables, and (iii) figures and to call attention to their significant parts. Report results concisely, using tables and figures to present important differences or similarities that cannot otherwise be presented or summarized in the text. The rationale and design of experiments should be made clear in the previous sections of the manuscript. Reserve subjective comments, interpretation, or reference to the previous literature for the "DISCUSSION". Number tables and figures in the order in which they are described and cited in the text. All tabular data should identify and report (i) either standard deviation values or standard errors of the means, (ii) the number of replicate determinations or human or animal subjects, and (iii) probability values and name(s) of statistical test(s) for reported differences. Restrict presentation of photo- and electron micrographs to

those essential to the results. If essential to the results, color can be published at the discretion of the Editor. (The cost for color in reprints, however, must be borne by the author. For cost estimates, contact the Central Office at 703-548-0066, or FAX 703-548-1883, e-mail publications@iadr.org.)

6)Discussion

Explain and interpret the results with a scientifically critical view of the previously published work in the field. Highlight the advances made by the new data. Indicate the limitations of the findings. State the conclusions of the report, and explain why they are merited by the data. This is the only proper section for subjective comments.

7)Acknowledgments

Recognize individuals who provided assistance to the project. Report all sources of grant and other support for the project or study, including funds received from contributors' institutions and commercial sources, and do not refer to a study being only partially funded by the cited sources. Consultancies and funds paid directly to investigators must also be listed, with statements such as "This investigation was supported in part by USPHS Research Grant DE-0000-00 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892".

8)References (maximum, 35)

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against the original documents and checked for correspondence between references cited in the text and listed in the "References" section.

Examples of correct forms of references are listed below. They are single-spaced here for illustration but should be double-spaced in the manuscript.

ARTICLES IN JOURNALS

1. Standard journal article

(List all authors, but if the number exceeds six, give six authors' names followed by *et al*.) West DJ, Snavely DB, Zajac BA, Brown GW, Babb CJ (1990). Development and persistence of antibody in a high-risk institutionalized population given plasma-derived hepatitis B vaccine. *Vaccine* 8:111-114.

2. Organization as author

The Royal Marsden Hospital Bone-Marrow Transplantation Team (1977). Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. *Lancet* 2:742-744.

3. No author given Coffee drinking and cancer of the pancreas (editorial) (1981). *Br Med J* 283:628.

4. Article in a foreign language

Massone L, Borghi S, Pestarino A, Picini R, Gambini C (1987). Localisations palmaires purpuriques de la dermatite herpetiforme. *Ann Dermatol Venereol* 114:1545-1547.

5. Volume with supplement

Magni F, Rossoni G, Berti F (1988). BN-52021 protects guinea pig from heart anaphylaxis. *Pharmacol Res Commun* 20(Suppl 5):75-78.

6. Issue with supplement

Gardos G, Cole JO, Haskell D, Marby D, Paine SS, Moore P (1988). The natural history of tardive dyskinesia. *J Clin Psychopharmacol* 8(4 Suppl):31S-37S.

7. Volume with part

Hanly C (1988). Metaphysics and innateness: a psychoanalytic perspective. *Int J Psychoanal* 69(Pt 3):389-399.

8. Issue with part

Edwards L, Meyskens F, Levine N (1989). Effect of oral isotretinoin on dysplastic nevi. *J Am Acad Dermatol* 20(2 Pt 1):257-260.

9. Issue with no volume

Baumeister AA (1978). Origins and control of stereotyped movements. *Monogr Am Assoc Ment Defic* (3):353-384.

10. No issue or volume

Danoek K (1982). Skiing in and through the history of medicine. *Nord Medicinhist Arsb*:86-100.

11. Pagination in Roman numerals

Ronne Y (1989). Ansvarsfall. Blodtransfusion till fel patient. *Vardfacket* 13:XXVI-XXVII.

12. Type of article indicated as needed

Spargo PM, Manners JM (1989). DDAVP and open heart surgery (letter). Anaesthesia 44:363-364. Fuhrman SA, Joiner KA (1987). Binding of the third component of complement C3 by *Toxoplasma gondii* (abstract). Clin Res 35:475A.

13. Article containing retraction

Shishido A (1980). Retraction notice: Effect of platinum compounds on murine lymphocyte mitogenesis (Retraction of Alsabti EA, Ghalib ON, Salem MH. In: *Jpn J Med Sci Biol* 1979; 32:53-65). *Jpn J Med Sci Biol* 33:235-237.

14. Article retracted

Alsabti EA, Ghalib ON, Salem MH (1979). Effect of platinum compounds on murine lymphocyte mitogenesis (Retracted by Shishido A. In: *Jpn J Med Sci Biol* 33:235-237, 1980). *Jpn J Med Sci Biol* 32:53-65.

15. Article containing comment

Piccoli A, Bossatti A (1989). Early steroid therapy in IgA neuropathy: still an open question (comment). *Nephron* 51:289-291. Comment on: *Nephron* 51:289-291, 1989.

16. Article commented on

Kobayashi Y, Fujii K, Hiki Y, Tateno S, Kurokawa A, Kamiyama M (1988). Steroid therapy in IgA nephropathy: a retrospective study in heavy proteinuric cases (see comments). *Nephron* 48:12-17. Comment in: *Nephron* 51:289-291, 1989.

17. Article with published erratum

Schofield A (1988). The CAGE questionnaire and psychological health (published *erratum* appears in *Br J Addict* 84:701, 1989). *Br J Addict* 83:761-764.

BOOKS AND OTHER MONOGRAPHS

18. Authored

Colson JH, Armour WJ (1986). Sports injuries and their treatment. 2nd rev. ed. London: Butterworth Heinemann.

19. Editor(s), compiler as author

Diener HC, Wilkinson M, editors (1988). Drug-induced headache. New York: Springer-Verlag.

20. Organization as author and publisher

Virginia Law Foundation (1987). The medical and legal implications of AIDS. Charlottesville, VA: The Foundation.

21. Chapters in a book

Weinstein L, Swartz MN (1974). Pathologic properties of invading microorganisms. In: Pathologic physiology: mechanisms of disease. Sodeman WA Jr, Sodeman WA, editors. Philadelphia: Saunders, pp. 457-472.

22. Conference Proceedings

Vivian VL, editor (1985). Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference on Child Abuse and Neglect, Mar 30-31, 1984, Chicago. Chicago, IL: American Medical Association.

23. Conference Paper

Harley NH (1985). Comparing radon daughter dosimetric and risk models. In: Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium, Oct 29-31, 1984, Knoxville, TN. Gammage RB, Kaye SV, editors. Chelsea, MI: Lewis Publishers, pp. 69-78.

24. Scientific and technical report

Akutsu T (1974). Total heart replacement device. Apr. Report No.: NIH-NHLI-69-2185-4. Bethesda, MD: National Heart and Lung Institute of the National Institutes of Health.

25. Dissertation

Youssef NM (1988). School adjustment of children with congenital heart disease (dissertation). Pittsburgh, PA: Univ. of Pittsburgh.

26. Patent

Harred JF, Knight AR, McIntyre JS, inventors (1972). Dow Chemical Company, assignee. Epoxidation process. US patent 3,654,317. Apr 4.

OTHER PUBLISHED MATERIAL

27. Newspaper article

Rensberger B, Specter B (1989). CFCs may be destroyed by natural process. *The Washington Post* Aug 7, Sect. A2, col. 5.

28. Audiovisual

AIDS epidemic: the physician's role (videorecording) (1987). Cleveland, OH: Academy of Medicine of Cleveland.

29. Computer file

Renal system (computer program) (1988). MS-DOS version. Edwardsville, KS: Medi-Sim.

30. Legal material

Toxic Substances Control Act: Hearing on S. 776 Before the Subcomm. on the Environment of the Senate Comm. on Commerce. 94th Cong., 1st Sess. 343 (1975).

31. Map

Scotland (topographic map) (1981). Washington: National Geographic Society .

32. Book of the Bible

Ruth 3:1-18. The Holy Bible. Authorized King James version (1972 ed.). New York: Oxford Univ. Press.

33. Dictionary and similar references

Ectasia. Dorland's illustrated medical dictionary. 27th ed. (1988). Philadelphia: Saunders, p. 527.

34. Classical material

The Winter's Tale: act 5, scene 1, lines 13-16. The complete works of William Shakespeare (1973). London: Rex.

UNPUBLISHED MATERIAL

35. In press

Lillywhite HB, Donald JA (1993). Pulmonary blood flow regulation in an aquatic snake. Science (in press).

9) Tables and 10) Figures (maximum, four total)

9)Tables

Type one table per page. In the order of mention in the text, number each table consecutively with Arabic numerals in the heading. In the heading, follow the table number with a brief descriptive title, generally highlighting the key result. Design tables to highlight key results and comparisons. Make every effort to make the presentation of data clear, simple, and uncluttered. As column headings, use accurate descriptors instead of symbols, acronyms, and abbreviations. To avoid overlong titles and cumbersome tables, use explanatory footnotes whenever possible. In the table or title, indicate the order of footnotes with superscript a,b,c,d,e,f, ... If needed in footnotes, cite the short form of references in parentheses. In tabular columns and the text, decimals less than unity must have the decimal point preceded by a zero. To ensure that the presentation is clear, report only the number of significant digits appropriate to the sensitivity and discrimination of the measure and the differences to be illustrated. Column headings should be simple and clear so that tables will be understandable without consultation of the text. Generally, column headings identify dependent variables, while independent variables are identified by row descriptors on the left. Tables will usually be printed either 3-1/4 or 7 inches wide.

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AUTHORITATIVE REFERENCES

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NOMENCLATURE

Authors should refer to the International System of Units (SI), D.T. Goldman and R.J. Bell, Eds., NBS Special Publication 330 (1981). This booklet is available from the US Dept. of Commerce, National Institute of Standards and Technology, Washington, DC 20234. Use of correct symbols includes m for milli-, for micro-, and L for liter (as in mL, L, etc.). Express grams as g, hours as hr, seconds as sec, and centrifugal force as g (e.g., 10,000 g). Use nm rather than Angstroms. Concentrations should be expressed as mol/L or mmol/L, etc. Insert leading zeros in all numbers less than 1.0 in the text, tables, and figures. Numbers of ten and fewer should be written out (e.g., ten subjects), except when indicating inanimate quantities (e.g., 10 mL), and numbers that are greater than ten should appear as digits. Always use digits to express dates, dimensions, degrees, doses, periods of time, percentages, proportions, ratios, sums of money, statistical results, weights, and measures, or to enumerate animals (but not people), culture cells and organisms, organs, and teeth. Leave a space between numbers and their accompanying units (e.g., 10 mg, not 10mg), and around the = and signs. Micro-organisms should be referred to in accordance with the International Rules of Nomenclature. When applicable, the nomenclature for bacteria presented in Bergey's Manual of Systemic Bacteriology (current edition) will be followed. The first reference to an organism by genus and species must be in full (e.g., Lactobacillus casei); subsequent mention may abbreviate genus (L. casei). When a common name of a bacterium or group is mentioned, do not italicize (e.g., "some lactobacilli" or "sanguis group streptococci").

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