UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

JIOVANNE RABELO NERI

INFLUÊNCIA DE ESTRATÉGIAS DE BIOMODIFICAÇÃO DENTINÁRIA NAS PROPRIEDADES FÍSICO-QUÍMICAS DE SISTEMAS ADESIVOS

FORTALEZA
2015

JIOVANNE RABELO NERI

INFLUÊNCIA DE ESTRATÉGIAS DE BIOMODIFICAÇÃO DENTINÁRIA NAS PROPRIEDADES FÍSICO-QUÍMICAS DE SISTEMAS ADESIVOS

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como um dos requisitos para a obtenção do título de Doutor em Odontologia.

Área de Concentração: Clínica Odontológica

Orientador: Prof. Dr. Sérgio Lima Santiago
Co-orientadora: Profa. Dra. Monica Yamauti

FORTALEZA
2015

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca de Ciências da Saúde

N364i Neri, Jiovanne Rabelo.

Influência de estratégias de biomodificação dentinária nas propriedades físico-químicas de sistemas adesivos/ Jiovanne Rabelo Neri. – 2015.

193 f.

Tese (Doutorado) – Universidade Federal do Ceará. Faculdade de Farmácia, Odontologia e Enfermagem. Programa de Pós-graduação em Odontologia, Fortaleza, 2015.

Área de concentração: Clínica Odontológica.

Orientação: Prof. Dr. Sérgio Lima Santiago.

1. Propriedades Físicas. 2. Dentina. 3. Catequina. 4. Compostos de Flúor. 5. Clorexidina. 6. Sistemas de Liberação de Medicamentos. I. Título.

CDD 617.6

JIOVANNE RABELO NERI

INFLUÊNCIA DE ESTRATÉGIAS DE BIOMODIFICAÇÃO DENTINÁRIA NAS PROPRIEDADES FÍSICO-QUÍMICAS DE SISTEMAS ADESIVOS

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como um dos requisitos para a obtenção do título de Doutor em Odontologia.

Aprovada em: 24/04/2015.

BANCA EXAMINADORA

Prof. Dr. Sérgio Lima Santiago (Orientador)
Universidade Federal do Ceará - UFC

Prof. Dr. Victor Pinheiro Feitosa
Universidade Federal do Ceará - UFC

Prof. Dr. Carlos José Soares
Universidade Federal de Uberlândia - UFU

Prof. Dr. Alessandro Dourado Loguércio
Universidade Estadual de Ponta Grossa - UEPG

Prof. Dr. Rafael Ratto de Moraes
Universidade Federal de Pelotas – UFPel

O presente trabalho não é simplesmente uma coleção de dados científicos, trata-se também de uma declaração de amor aos meus pais, Neri e Ilnar, e aos meus irmãos, Eugenie, Niskiêr e Pascoal.

AGRADECIMENTOS ESPECIAIS

A **Deus**, por todas as oportunidades, realizações pessoais e profissionais que me foram dadas.

Aos meus sobrinhos **Isadora, Cecília e Arthur**, pequenos presentes que Deus enviou para alegrar mais ainda nossa família.

Ao meu grande amor **Eliza Barros**, uma mulher guerreira e adorável, que sempre me apoiou e me incentivou.

Ao grande professor, mestre, mentor e AMIGO **Sérgio Santiago**, uma pessoa a quem aprendi, ao longo desses anos, a admirar pela capacidade, pela forma cuidadosa como trata as pessoas, e principalmente, pelo dom de enxergar o simples.

A Profa. Dra. Monica Yamauti, que não poupou esforços para meu crescimento profissional e de quem recebi ajuda fundamental para o desenvolvimento desta tese.

Aos meus irmãos inoxidáveis: **Gledson Barros, Yuri Castro, Afranio Naninha** por serem especiais. Nossas amizades serão eternas.

Aos nobres colegas de doutorado, **Lívia de Oliveira Barros, Fábio Wildson Gurgel Costa, Kátia Linhares Lima Costa, Marcelo Ferraro Bezerra e Virgínia Régia Souza da Silveira**, pela ótima convivência durante a pós-graduação. Fico extremamente envaidecido de ter feito parte de uma turma com tantos expoentes. OBRIGADO.

Aos colegas de pós-graduação **Nadine Guimarães, Cecília Atem e Jacqueline Nojosa**, com os quais desfrutei valores como dignidade, honestidade, lealdade e respeito. Este trabalho tem um pouco de vocês em cada palavra

Aos alunos de graduação **Marcelo Sidou, Mirele Nobre e Edson Cetira**. Tenho certeza de que serão, em breve, colegas de profissão acima da média. Continuem na caminhada rumo ao sucesso.

AGRADECIMENTOS

À Universidade Federal do Ceará, na pessoa do seu Magnífico Reitor **Prof. Dr. Jesualdo Pereira Farias**.

À Faculdade de Farmácia, Odontologia e Enfermagem, na pessoa de sua diretora **Profa. Dra. Maria Goretti Rodrigues de Queiroz.**

Aos professores do Programa de Pós-Gradação em Odontologia da Universidade Federal do Ceará, em nome da coordenadora **Profa. Dra. Lidiany Karla Azevedo Rodrigues**.

Ao **Prof. Dr. Rinaldo dos Santos Araújo** e a aluna de pós-graduação **Amanda Pontes Maia Pires**, pelo suporte técnico e científico nos laboratórios do Instituto Federal de Educação, Ciência e Tecnologia do Estado do Ceará (IFCE).

À Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), pela concessão de bolsa de auxílio financeiro nos dois primeiros anos de doutorado.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), pela concessão de bolsa de auxílio financeiro nos dois últimos anos de doutorado.

“Pessoas quietas possuem mentes barulhentas.”

(Stephen Hawking)

RESUMO

Proporcionar maior durabilidade aos procedimentos adesivos quando executados sobre a dentina é considerado um desafio. Portanto, o uso de agentes bioativos pode ser uma estratégia promissora para preservar a camada híbrida. Dessa forma, a presente tese foi constituída por cinco capítulos que objetivaram, respectivamente: 1) Avaliar o efeito de soluções de desinfecção cavitária bioativas na resistência de união de sistema adesivo autocondicionante à dentina após termociclagem; 2) Avaliar a efetividade da biomodificação dentinária com epigalocatequina-3-galato (EGCG) na resistência de união de sistema adesivo autocondicioante ao longo do tempo; 3) Avaliar a influência da incorporação de EGCG nas propriedades físico-químicas de um sistema adesivo autocondicionante; 4) Promover o desenvolvimento e a caracterização de partículas poliméricas para liberação controlada de epigalocatequina-3-galato, usando dois tipos de de ácido polilático glicólico (PLGA); e 5) Avaliar o efeito da incorporação de micropartículas de PLGA carregadas com EGCG nas propriedades físico-químicas de sistema adesivo convencional de 2 passos. Como abordagens metodológicas foram realizados 5 estudos *in vitro*. No Capítulo 1, a superfície dentinária de 18 dentes foram tratadas com água destilada, fluoreto de sódio (NaF) ou clorexidina (CHX). Espécimes em forma de palito foram confeccionados e submetidos ao teste de resistência de união (μTBS), após 24 horas e 60.000 ciclos térmicos. No Capítulo 2, a superfície dentinária de 27 dentes foram tratadas com água destilada, EGCG ou CHX. Espécimes em forma de palito foram confeccionados e submetidos ao teste de μTBS, após 24 h, 6 meses e 12 meses de armazenamento. Para o Capítulo 3, o EGCG foi incorporado diretamente, em concentrações de 0,01% e 0,1%, a um sistema adesivo autocondicionante de 1 passo e, em seguida, foram realizados os testes de sorção (S) e solubilidade (SL), grau de conversão (GC) e resistência flexural (RF). No Capítulo 4, micropartículas foram desenvolvidas a partir de uma técnica de atomização por secagem e, em seguida, foram determinadas o tamanho e a morfologia das partículas, o rendimento, a eficácia de encapsulação e carregamento de EGCG e a liberação da catequina pelas micropartículas. Por fim, no Capítulo 5, além da incorporação direta de EGCG a 0,01% e 0,1%, foram incorporadas também micropartículas de dois tipo de PLGA (PLGA 50:50 E PLGA 75:25) carregadas com EGCG, na concentração de 0,5%, 1% e 2%. Inicialmente foi realizado o ensaio de liberação de EGCG e, em seguida, foram avaliados o GC, a RF, o módulo de elasticidade (ME), a S, a SL e a μTBS. Os dados obtidos foram submetidos a teste de Análise de Variância e eventuais diferenças estatísticas foram analisadas pelo teste de Holm-Sidak. O nível de significância adotado foi de 5%. Os resultados mostraram que o NaF manteve

a resistência de união após a termociclagem (p=0,336) (Capítulo 1). Os grupos tratados com EGCG e CHX não interferiram na resistência de união após 24 h (p>0,05), e mantiveram a resistência de união após 6 e 12 meses (p<0,05) (Capítulo 2). Não houve diferença estatística entre os valores de WS, GC e RF quando os grupos foram comparados (p>0,05). Contudo, a incorporação de EGCG reduziu significativamente a SL (p<0,05) (Capítulo 3). Não houve diferença estatística entre os grupos em relação aos valores de GC, ME, S, SL e μTBS imediata. (p>0,05). Os valores de RF foram significativamente elevados pela incorporação de micropartículas poliméricas carregadas com EGCG na concentração de 1% (p<0.05). As micropartículas confeccionadas com PLGA 50:50 e PLGA 75:25 apresentaram um padrão de liberação pulsátil (Capítulo 4). A incorporação de micropartículas poliméricas carregadas com EGCG ao sistema adesivo obteve a maior taxa de liberação de EGCG entre todos os grupos (p<0,05) (Capítulo 5). Portanto, conclui-se que as estratégias de biomodificação dentinária além de não prejudicarem as propriedades físicas dos sistemas adesivos estudados, podem também manter as interfaces de união mais estáveis ao longo do tempo.

Palvras-chave: Propriedades físicas; Dentina; Catequina; Compostos de flúor; Clorexidina; Sistemas de liberação de medicamento

ABSTRACT

Provide greater durability to the bonding procedures when performed on the dentin is considered a challenge. Therefore, the use of bioactive agents may be a promising strategy to preserve the hybrid layer. Thus, this thesis consisted of five chapters that aimed, respectively: 1) To evaluate the effect of sodium fluoride on the resin-dentin bond strength of a self-etch adhesive after thermal cycles; 2) To evaluate the effectiveness of dentin biomodification with epigallocatechin-3-gallate (EGCG) on the resin-dentin bonds overtime; 3) To evaluate the influence of EGCG incorporation on the physicochemical properties of a methacrylate-based dental adhesive; 4) To develop and characterize of poly (lactide-co-glycolide) acid (PLGA) micropartciles for controlled release of epigallocatechin-3-galate (EGCG), using two types of PLGA; 5) To evaluate the EGCG-load PLGA microparticules incorporation on the physicochemical properties of a two-step etch-and-rinse adhesive system, and the release rate of EGCG. As methodological approaches were performed 5 *in vitro* studies. In the Article 1, dentin surface of 18 teeth were treated with distilled water, 2% chlorhexidine digluconate solution (CHX) or 1.23% sodium fluoride solution (NaF). Bonded sticks were obtained and submitted to bond strength test (μTBS), after 24 h and 60.000 thermal cycles. In Article 2, dentin surface of 27 teeth were treated with distilled water, or EGCG, or CHX. Bonded sticks were obtained and submitted to bond strength test (μTBS), after 24 h, 6 and 12 months of storage. To Article 3, EGCG was added to one-step self-etch adhesive, except in control group, to obtain concentrations of 0.01% w/w and 0.1% w/w of EGCG-doped adhesives. Then, water sorption (WS), solubility (SL), degree of conversion (DC) and flexural strength (FS) tests were performed. To Article 4, microparticles were developed from a spray drying technique and then were determined size and particle morphology, yield, efficacy of encapsulation, drug loading and and EGCG release. Finally, in Article 5, beyond the concentrations of 0.01% w/w and 0.1% w/w of EGCG-doped adhesives, were also incorporated EGCG-load polymeric microparticles at 1%. Then, DC, FS, elastic modulus (E), WS, SL, DC, μTBS and EGCG release. Data were submitted to ANOVA and any statistical differences were analyzed by Holm-Sidak test. The significance level was 5%. The results showed that the NaF maintained bond strength after thermocycling ($p = 0.336$) (Chapter 1). Groups treated with EGCG and CHX did not affect the bond strength after 24 h ($p > 0.05$), and maintained the bond strength after 6 and 12 months ($p < 0.05$) (Chapter 2). There was no statistical difference between the WS, GC and RF values when the groups were compared ($p > 0.05$). However, the incorporation of EGCG reduced SL values ($p < 0.05$) (Chapter 3). There was no significant difference in the DC, E, WS SL and μTBS values among all groups ($p > 0.05$). FS values were significantly increased by

incorporating polymer microparticles loaded with EGCG at 1% ($p<0.05$). The microparticles made from PLGA 50:50 and 75:25 PLGA showed a pattern of pulsatile release (Chapter 4). Polymeric microparticles had the highest EGCG release rates, when compared with other groups ($p<0.05$) (Chapter 5). Therefore, it is concluded that the dentin biomodification strategies besides not impair the physical properties of the adhesive systems may also maintain more stable bonding interface over time.

Key words: Physical properties; Dentin; Catechin; Fluorine compounds; Chlorhexidine; Drug delivery system

SUMÁRIO

Introdução

1 INTRODUÇÃO

Os sistemas adesivos evoluíram consideravelmente, desde a sua introdução há mais de 50 anos, e influenciaram indubitavelmente os rumos da Odontologia restauradora atual (CARDOSO *et al*., 2011). Todavia, proporcionar maior durabilidade aos procedimentos adesivos, principalmente quando executados sobre a dentina, ainda é considerado um desafio (DE MUNCK *et al*., 2005, VAN MEERBEEK *et al*., 2011).

A dentina é um tecido mineralizado complexo disposto em uma estrutura tridimensional repleta de túbulos, que se estendem da polpa à junção amelo-dentinária (GARBEROGLIO; BRÄNNSTRÖM, 1976; BEDRAN-RUSSO *et al*., 2014). Aproximadamente, 50% do volume da dentina é composto por minerais, o restante é constituído por colágeno do Tipo I e proteínas não colágenas (30%), e água (20%) (MARSHALL, 1993; MARSHALL *et al*., 1997; TJÄDERHANE *et al*., 2012; TJÄDERHANE *et al*., 2013a). A execução de procedimentos restauradores adesivos sobre o substrato dentinário é considerada crítica devido à sua heterogeneidade e complexa morfologia (PASHLEY; CARVALHO, 1997).

Durante a realização do procedimento adesivo, a superfície e a subsuperfície da dentina são desmineralizadas pelo condicionamento ácido, dos sistemas adesivos convencionais ou pelos monômeros resinosos acídicos, dos sistemas adesivos autocondicionantes (TJÄDERHANE *et al*., 2013b). Contudo, os monômeros resinosos não são capazes de envolver totalmente as fibrilas colágenas, deixando-as expostas, principalmente, na base da camada híbrida. As áreas incompletamente infiltradas por monômeros dentro da camada híbrida podem ser consideradas o ponto de partida para o processo de degradação dos componentes resinosos e das fibrilas colágenas que compõem a camada híbrida (TAY & PASHLEY, 2003; PASHLEY *et al*., 2004).

A biodegradação das fibrilas colágenas que não foram completamente envolvidas ocorre através da ação de proteases presentes na matriz extracelular dentinária conhecidas como metaloproteinases da matriz (MMPs) (PASHLEY *et al*., 2004; HEBLING *et al*., 2005; OSORIO *et al*., 2013; TOLEDANO *et al*., 2013, MAZZONI *et al*., 2013; MAZZONI *et al*., 2015) e cisteínas catepsinas (NASCIMENTO *et al*., 2011; TJÄDERHANE *et al*., 2013; BEDRAN-RUSSO *et al*., 2014; VIDAL *et al*., 2014a). As MMPs são endopeptidases, zinco e cálcio dependentes, que contribuem para a organização e para a mineralização da matriz dentinária (TJÄDERHANE *et al*., 1998; VAN STRIJP *et al*., 2003). Essas enzimas são depositadas na dentina de forma inativa, e são conhecidas nessa etapa como zimogênio (VISSE; NAGASE, 2003). A latência das MMPs é decorrente da existência de um propeptídeo contendo

um resíduo de cisteína conservado, unido ao íon zinco do seu sítio catalítico (NAGASE; WOESSNER, 1999). O rompimento da ligação zinco-cisteína pode ocorrer devido a fatores químicos, como o baixo pH dentinário decorrente da aplicação de ácido fosfórico ou de monômeros acídicos (NISHITANI *et al*., 2006; OSORIO *et al*., 2011).

Por outro lado, as cisteínas catepsinas (CTs), enzimas pertencentes à família das papaínas, são expressas por odontoblastos humanos e por outras células da polpa, e podem facilmente alcançar os túbulos dentinários e porções mais profundas da dentina (TERSARIOL *et al*., 2010; TURK *et al*., 2012). O colágeno íntegro pode ser degradado pela CT-K, em regiões específicas da sua tripla hélice (BRÖMME; OKAMOTO, 1995; HOU *et al*., 2002), enquanto, o colágeno desnaturado é degradado por vários outros tipos de CTs (LI *et al*., 2002). Essas enzimas são encontradas tanto em dentina sadia como cariada, contudo, a atividade das CTs é aumentada de forma significativa em dentina profunda e cariada, especialmente em pacientes jovens (NASCIMENTO *et al*., 2011; VIDAL *et al*., 2014b). Levando em consideração que a maioria das restaurações é executada em dentina profunda e cariada, é possível que níveis elevados de atividade de CTs também estejam presentes nos túbulos dentinários, durante a execução do procedimento adesivo, o que fatalmente levaria à degradação gradual das fibrilas colágenas da camada híbrida (TJÄDERHANE *et al*., 2013b). Desta forma, novas abordagens devem ser desenvolvidas para preservar a camada híbrida e garantir a longevidade dos procedimentos adesivos em dentina.

A biomodificação dentinária é uma estratégia biomimética que objetiva melhorar as propriedades biomecânicas e bioquímicas do tecido dentário duro (BEDRAN-RUSSO *et al*., 2014). A aplicação de agentes bioativos, sintéticos ou naturais, durante os procedimentos adesivos pode modificar a estrutura do colágeno Tipo I, através da formação de ligações cruzadas, aumentando assim a resistência à tração e o módulo de elasticidade do colágeno (AL-AMMAR; DRUMMOND; BEDRAN-RUSSO, 2009). Por outro lado, a maioria das estratégias de biomodificação atuam reduzindo a atividade proteolítica das enzimas que degradam a matriz extracelular, promovendo a remineralização da dentina e prevenindo a ocorrência de lesões cariosas (BEDRAN-RUSSO *et al*., 2014).

Os fluoretos são agentes bioativos amplamente utilizados na prática odontológica devido a sua comprovada ação anticárie (TENUTA; CURI, 2010; TENUTA *et al*., 2008). Contudo, nos últimos anos o fluoreto de sódio tem chamado a atenção por apresentar também a capacidade de prevenir a degradação do colágeno da camada híbrida através da inibição da atividade da MMP-2 e da MMP-9 (MEI *et al*., 2012; KATO *et al*., 2014). Adicionalmente, as soluções fluoretadas podem melhorar as propriedades biomecânicas da dentina através do seu

efeito remineralizador (ITOTA *et al*., 2002). Desta forma, o uso do fluoreto de sódio pode resultar na estabilização das interfaces adesivas (NERI *et al*., 2011).

Outro agente bioativo sintético que tem ganhado notoriedade perante a comunidade científica é o digluconato de clorexidina (BRESCHI *et al*., 2008; BRESCHI *et al*., 2009). Além de ser um excelente agente antimicrobiano, a clorexidina também é um potente inibidor de MMP-2, MMP-8 e MMP-9 (GENDRON *et al*., 1999; ZHOU *et al*., 2011; TOLEDANO *et al*., 2012), e CT-B, CT-L e CT-K (SCAFFA *et al*., 2012). O digluconato de clorexidina pode atuar de duas maneiras para inativar as MMPs: através da quelação com os íons zinco ou mediante a interação com grupos sulfidril e/ou cisteínas presentes nos sítios catalíticos das MMPs ativas (GENDRON *et al*., 1999). O emprego do digluconato de clorexidina tem apresentado resultados promissores em estudos *in vivo* e *in vitro*, confirmando a manutenção da resistência de união ao longo do tempo (CARRILHO *et. al*, 2007a; CARRILHO *et al*., 2007b; LOGUERCIO *et al*., 2009; CAMPOS *et al*., 2009; STANISLAWCZUK *et al*., 2009; BRESCHI *et al*., 2010; SANTIAGO *et al*., 2013). Todavia, por apresentar reações inflamatórias e necrose tecidual (DE SOUZA *et al*., 2007; FARIA *et al*., 2007), tem-se procurado substâncias mais biocompatíveis e provenientes de produtos naturais (SANTIAGO *et al*., 2013).

O epigalocatequina-3-galato (EGCG) é o principal polifenol encontrado no chá verde (*Camellia sinesis*), correspondendo a mais de 65% das catequinas encontradas nesse chá (DEMEULE *et al*., 2000). Esse polifenol despertou grande interesse científico devido ao seu potencial antimutagênico e anticancerígeno (CAO & CAO, 1999; GARBISA *et al*., 2001; SEN *et al*., 2009). O epigalocatequina-3-galato apresentou baixa toxicidade e propriedades anti-inflamatórias quando em contato com células pulpares e possui comprovada capacidade de inibir a ação de MMP-2 e MMP-9 (DEMEULE *at al*., 2000; GARBISA *et al*., 2001; DELL'AICA *et al*., 2007; VIDAL *et al*, 2014), e CTs (DEVIKA; PRINCE, 2008, VIDAL *et al*., 2014). Estudos prévios demonstraram que o epigalocatequina-3-galato se liga ao colágeno através de pontes de hidrogênio, e promove o aumento do número de ligações cruzadas inter e intra-fibrilares, prevenindo, portanto, o livre acesso das colagenases aos sítios específicos de degradação na cadeia de colágeno (JACKSON *et al*., 2010; VIDAL *et al*, 2014; BEDRAN-RUSSO *et al*, 2014).

O epigalocatequina-3-galato tem sido utilizado na Odontologia adesiva em diferentes estratégias (pré-tratamento dentinário e incorporado ao sistema adesivo) e em diferentes concentrações (0,01%, 0,02%, 0,03%, 0,1%, 0,5%), resultando na manutenção da resistência de união após 6 meses de armazenamento dos espécimes (DU *et al*., 2012, SANTIAGO *et al*., 2013). Embora a incorporação de epigalocatequina-3-galato aos sistemas adesivos tenha

demonstrado resultados promissores, há uma preocupação em relação à sua biodisponibilidade dentro da camada híbrida. Pallan *et al*. (2012) observaram uma alta taxa de liberação de EGCG, nas primeiras 24 horas de armazenamento em água destilada, seguida de uma redução significativa na taxa de liberação até o 28º dia. Portanto, o tempo de permanência do EGCG na camada híbrida provavelmente foi curto devido solubilidade da molécula em água (PALLAN *et al*., 2012). Esse inconveniente pode ser contornado a partir de métodos de liberação controlada de fármacos (LIANG *et al*., 2005; GAIGNAUX *et al*., 2012).

A liberação lenta e duradoura do epigalocatequina-3-galato pode ser obtida a partir do seu encapsulamento em micropartículas de ácido poli-láctico-co-glicólico (PLGA) (ANDERSON & SHIVE, 1997; MATSUMOTO *et al*., 2005). O PLGA é considerado o material padrão-ouro na liberação controlada de fármacos, devido a sua incomparável biodegradação e biocompatibilidade (JAIN, 2000; TAMBER *et al*., 2005). A liberação dos fármacos ocorre a partir do contacto das micropartículas com os fluidos orgânicos aquosos (GAIGNAUX *et al*., 2012). A água penetra nas micropartículas, promove a degradação da cadeia do polímero e a dissolução do fármaco. Uma vez dissolvido, o fármaco começa a difundir-se para o fluido circundante através da rede de polímero degradado (LI; ROUAND; PONCELET, 2008). Apesar dos benefícios comprovados da liberação controlada de fármacos nas ciências médicas, pouco se sabe a respeito da sua utilização em Odontologia adesiva.

Nesse contexto, o uso de agentes bioativos pode significar um importante avanço na tentativa de se obter maior estabilidade dos componentes da camada híbrida, tornando os procedimentos adesivos menos susceptíveis à degradação.

Proposição

2 PROPOSIÇÃO

A presente tese será apresentada em capítulos, tendo como objetivos:

Capítulo 1: Avaliar a influência do fluoreto de sódio na resistência de união de sistema adesivo autocondicionante à dentina após termociclagem.

Capítulo 2: Avaliar a efetividade da biomodificação dentinária com epigalocatequina-3-galato na resistência de união de sistema adesivo autocondicioante ao longo do tempo.

Capítulo 3: Avaliar a influência da incorporação de epigalocatequina-3-galato nas propriedades físico-químicas de um sistema adesivo autocondicionante.

Capítulo 4: Promover o desenvolvimento e a caractericação de partículas poliméricas para liberação controlada de epigalocatequina-3-galato, usando dois tipos de PLGAs.

Capítulo 5: Avaliar o efeito da incorporação de micropartículas de ácido polilático coglicólico carregadas com epigalocatequina-3-galato nas propriedades físico-químicas de sistema adesivo convencional de 2 passos, e a taxa de liberação de epigalocatequina-3-galato.

Capítulos

3 CAPÍTULOS

REGIMENTO INTERNO

A presente tese está baseada no Artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de pesquisas envolvendo seres humanos, os projetos de pesquisa referentes aos trabalhos desenvolvidos nos capítulos 1, 2 e 5 foram submetidos à apreciação do Comitê de Ética em Pesquisas da Universidade Federal do Ceará, tendo sido aprovados (Anexos B, D e G). Desta forma, a tese é composta por cinco artigos, sendo um artigo publicado e quatro a serem submetidos para publicação em periódicos científicos, conforme descrito abaixo:

Capítulo 1: Does sodium fluoride maintain resin–dentin bond strength after thermal stressing? O presente artigo será submetido à publicação na revista "*European Journal of Oral Sciences*".

Capítulo 2: Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: twelve-month results. O presente artigo será submetido à publicação na revista "*Journal of Biomedical Materials Research Part B: Applied Biomaterials*".

Capítulo 3: Physicochemical properties of a methacrylate-based dental adhesive incorporated with epigallocatechin-3-gallate. O presente artigo foi submetido e publicado na revista "*Brazilian Dental Journal*".

Capítulo 4: Development and characterization of PLGA microparticles for controlled release of epigallocatechin-3-galate. O presente artigo será submetido à publicação na revista "*International Journal of Pharmaceutics*".

Capítulo 5: Physicochemical properties and pattern of drug release of etch-and-rinse adhesive system incorporated with catechin-loaded polymeric microparticles. O presente artigo será submetido à publicação na revista "*Journal of Dentistry*".

Capítulo 1

3.1 CAPÍTULO 1

Does Sodium fluoride maintain resin–dentin bond strength after thermal stressing?

Running title: Sodium fluoride maintain MTBS

Authors: Jiovanne Rabelo NERI[1,2], Jacqueline de Santiago NOJOSA[2], Monica YAMAUTI[3], Sérgio Lima SANTIAGO[2]

Affiliations:
[1]*Graduate School of Dentistry, University of Fortaleza, Fortaleza, Ceará, Brazil; PhD student, Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.*
[2]*Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.*
[3]*Department of Restorative Dentistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

*Corresponding author:
Sérgio Lima Santiago
Rua Monsenhor Furtado S/Nº
60430-355
Fortaleza, CE - Brazil
E-mail: sergiosantiago@ufc.br
Tel: +558588242704
Fax: +558533668232

Neri J.R., Nojosa J.S, Yamauti M., Santiago S. L. Does Sodium fluoride maintain resin–dentin bond strength after thermal stressing? Eur J Oral Sci

ABSTRACT

This study evaluated the effect of sodium fluoride on the resin-dentin bond strength of a self-etch adhesive after thermal cycles. Eighteen human third molars were prepared to expose a flat dentin surface and were divided into 3 groups (*n*=6) according to the cavity cleaning solutions, as follows: distilled water, 2% chlorhexidine digluconate solution (CHX) or 1.23% sodium fluoride solution (NaF). Solutions were rubbed for 60 seconds on dentin surfaces, followed by bonding with Clearfil SE Bond, and 5-mm-thick resin crown build-up. Bonded teeth were stored in distilled water for 24 hours and then longitudinally sectioned to obtain bonded sticks. Half of the specimens were immediately tested in tension at 0.5 mm/min, while the remaining specimens were tested after 60.000 thermal cycles. Data were analyzed using Two-way ANOVA and the Holm-Sidak method. There was no significant difference between the groups after 24 hours ($p>0.05$). Thermocycling resulted in significant bond strength reduction for distilled water and CHX ($p<0.05$). When 24 hours values were compared to thermocycling, NaF maintained its bond strength ($p>0.05$), while significant reductions in bond strength were observed for distilled water and CHX ($p<0.05$). Pretreatment with NaF preserved the bonding of Clearfil SE Bond to dentin after 60.000 thermal cycles.

Key words: Dentin-Bonding Agents; Sodium Fluoride; Self-etch adhesive; Dentin bond strength.

*Corresponding author:
Sérgio Lima Santiago
Rua Monsenhor Furtado S/Nº
60430-355
Fortaleza, CE - Brazil
E-mail: sergiosantiago@ufc.br
Tel: +558588242704
Fax: +558533668232

INTRODUCTION

Self-etch adhesives contain acidic monomers that simultaneously dissolve and infiltrate the smear layer, smear plugs and hydroxyapatite to generate a hybrid layer (1). However, the use of self-etching adhesive systems on smear layers has been considered a controversial matter (2). Self-etching primers may not be able to penetrate through thick smear layers (3). The acidity of the primer could also be buffered by the mineral components of the smear layer to the extent that the potential for primer penetration into the underlying sound dentin might be reduced, resulting in gaps in adhesive restorations (3). Thus, the pretreatment of the smear layer can be an alternative for improving the longevity of the restorative procedures (4,5).

The use of cavity cleaning solutions prior to restorative procedures may totally or partially remove the smear layer (6). Chlorhexidine digluconate is a non-demineralizing agent that acts via the mechanical action of washing and scrubbing, which may promote changes in the smear layer without exposing opened dentinal tubules (6). It has been widely used as an antimicrobial agent due to its broad spectrum of action against Gram-negative and Gram-positive bacteria, particularly Streptococcus mutans (7). Additionally, chlorhexidine has shown to preserve dentin bond strength via the inhibition of matrix metalloproteinases (MMPs) and cysteine cathepsins (8-11).

On the other hand, when dentin is cleaned with 1.23% acidic sodium fluoride solution, the smear layer and smear plug are completely removed, exposing dentinal tubules (6). Thus, the removal of smear layer provide direct contact of sel-etch primers with dentin, and can influence the formation of compact hybrid layer (3). Recently, sodium fluoride has demonstrated its efficacy on the inhibition of MMP action, which suggests its potential to preserve the resin-dentin interface (12, 13). Although fluoride solutions could present beneficial effects in preserving bonded interfaces, little is known about the use of 1.23% sodium fluoride solution, as a cavity cleanser associated with self-etch adhesives.

The aim of this study was to evaluate the influence of sodium fluoride on the resin-dentin bond strength of a self-etching adhesive after thermal cycles. The tested null hypotheses were that 1) there will be no differences in immediate bond strength caused by different pretreatments and 2) there will be no differences in the bond strength between the 24-h period and after thermocycling for all groups.

MATERIALS AND METHODS

Eighteen unerupted third molars were collected after patients' informed consents had been obtained under a protocol (#35/12) that was reviewed and approved by the local Ethics Committee. Selected teeth were stored in 0.01% thymol solution and used within one month after extraction. Occlusal enamel was removed using a #120-grit silicon carbide (SiC) paper mounted to an electric polishing machine (Aropol 2V; Arotec, São Paulo, SP, Brazil) to expose a flat coronal dentinal surface. The dentinal surface was prepared with #600-grit SiC paper under copious water for 60 s to standardize the smear layer.

The teeth were randomly allocated using Excel software (Excel 2003, Microsoft Corporation, Redmond, WA, USA) into 3 groups (n=6) according to the pre-treatment solution used prior to adhesive application. Dentinal surfaces of all teeth were dried with oil/water-free air for 10 s and treated with 10 µL of distilled water (pH 7.40), 2% chlorhexidine digluconate solution (pH 7.55) (CHX) (FGM, Joinville, SC, Brazil - batch #210211) or 1.23% sodium fluoride solution (pH 3.60) (NaF). The solutions were rubbed for 60 s, and the excess of each solution was removed with absorbent paper.

The two-step self-etch adhesive system, Clearfil SE Bond (Kuraray Medical, Tokyo, Japan), was applied according to the manufacturer's instructions (Table 1) and light-cured with Variable Intensity Polymerizer, VIP Junior (500 mW/cm^2, Bisco Inc., Schaumburg, IL, USA). Five 1-mm-thick resin composite increments were built up (Filtek Z250; 3M ESPE, St. Paul, MN, USA - batch #1117600319). Each increment was light cured for 20 s. The bonded teeth were stored in distilled water at 37°C for 24 h.

After storage, the prepared teeth were longitudinally sectioned in both “x” and “y” directions across the bonded interface using a diamond saw in a Labcut 1010 (Extec, Enfield, CT, USA) under water cooling to obtain sticks with cross-sectional area of approximately 1.0 mm^2. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm and recorded for the subsequent calculation of bond strength values. Half of the sectioned sticks for each tooth were randomly selected using the Excel software (Excel 2003, Redmond, WA, USA) and immediately tested under tension. The remaining sticks were submitted at 60.000 thermal cycles (THE-1100; SD Mechatronik GMBH, Feldkirchen-Westerham, Germany) in distilled water at 5 ± 2°C and 55 ± 2°C baths, with a dwell time of 60 s in each bath. Thermocycling took approximately 3 months (approximately 20,000 cycles per month).

For the microtensile test, each bonded stick was attached with cyanoacrylate glue (Super Bonder Gel, Loctite, São Paulo, SP, Brazil) to a modified Geraldeli testing apparatus (Odeme Biotechnology, Joaçaba, SC, Brazil) and subjected to a tensile force at 0.5 mm/min in a universal testing machine (Instron 3345; Instron Inc., Canton, MA, USA). The load at fracture was used to calculate bond strength (MPa).

The failure mode was evaluated using a stereoscope at 80X magnification (StereoZoom® Leica S8 APO, Leica Microsystems, Wetzlar, Germany) and classified as cohesive when the fracture occurred exclusively within the dentin (CD) or resin composite (CR); adhesive (A) when the failure was at the dentin/resin interface or mixed (M) when two modes of failure (adhesive and cohesive) occurred simultaneously.

A Shapiro-Wilk test was applied to all groups to analyze the normal distribution of errors and the Bartlett test was applied to test for homoscedasticity. After a normal distribution was confirmed, data were analyzed with a two-way ANOVA (pretreatments and aging) and the Holm-Sidak method was used for *post hoc* comparisons. Statistical procedures were performed with the Sigmastat 3.5 for Windows (Systat Software Inc., San Jose, CA, USA) statistical program software. The level of significance was set at $p<0.05$. Teeth were used as a statistical unit and the number of prematurely debonded specimens was recorded, although this was not included in the analysis.

RESULTS

Bond strength results are shown in Table 2. The results were affected by treatment ($p=0.020$; $F=4.615$) and aging ($p<0.001$; $F=13.161$). Interactions were not statistically significant ($p=0.815$; $F=0.206$).

After 24 h of storage, no significant difference was observed among the bond strength values of all groups ($p>0.05$). After thermocycling, NaF had the highest bond strength values, when compared with CHX ($p=0.025$) and distilled water ($p=0.048$). When 24-h values were compared to thermocycling, NaF maintained its bond strength ($p=0.129$), whereas significant reductions in bond strength were observed for distilled water ($p=0.029$) and CHX ($p=0.025$) (Table 2).

Table 3 summarizes the percentage of failure modes of the debonded specimens. Most failures were mixed in all tested groups for all tested conditions. There was an increase in the number of premature failures for all groups, mainly to distilled water and CHX after thermocycling.

DISCUSSION

The smear layer represent a great challenge for the interaction of adhesive systems and prepared tooth surfaces (14). Once that the thickness and structure of smear layer may interfere in the demineralizing potential of adhesives systems, thus decreasing the resin-dentin bond strength (15-17). However, in the present study the presence of a smear layer did not seem to affect the immediate bond strength of self-etch adhesive to dentin (Table 2), corroborating with others studies (18-20). This fact could be explained due to the use of 600-grit SiC paper to standardize smear layer, resulting in the formation of thin smear layer (thickness ≈ 1 μm) (21, 22). When Clearfill SE Bond was applied on thin smear layer besides to demineralize and completely infiltrate in the smear layer (hybridized smear layer) also forms a true hybrid layer with the superficial peritubular dentin (3).

Previous studies have reported that the application of chlorhexidine prior to the self-etch adhesive had no adverse effects on immediate adhesive bonds in dentin (23-27). The present study results corroborate the results from previous ones. Nevertheless, other researchers have suggested that such use of chlorhexidine prior to the application of self-etch adhesives should be avoided because of potential interactions among chlorhexidine and the adhesive components (28-30). Chlorhexidine may decrease wettability and the level of dentin conditioning of self-etching adhesives, which could interfere with the stability of the hybrid layer (28).

The results of the current study show that the application of NaF did not affect the immediate resin-dentin bond strength in the same way that the distilled water and CHX. Thereby, failed to reject the first hull hypothesis. Although, sodium fluoride solution with low pH (pH= 3.6) removes the smear layer and exposes dentin tubules (6), the thickness of true hybrid layer formed on dentin without smear layer is similar to dentin with thin smear layer, approximately 0.5 μm thick (3). According to TAY *et al.* (20), the immediate bond strength between mild-self etch adhesive system and dentin with and without smear layer were not significantly different.

On the other hand, the second null hypothesis was rejected because significant reductions in bond strength were observed for distilled water and CHX when the 24-h were compared to thermocycling (Table 2). The stability of the bonded interface relies on the creation of a compact and homogenous hybrid layer (10). Nevertheless, mild self-etch adhesives, as the one used in this study, applied on thin smear layer increased the formation of an interfacial gap (18), leading to the possible separation of the hybridized smear layer from the true hybrid layer (20). In a recent study, a mild self-etch adhesive failed predominantly under the hybrid layer

after aging, which may have been the result of insufficient penetration of self-etch primers in the dentin (32). SABOIA *et al*. (33) speculated that during thermocycling the repetitive contraction/expansion stresses generated at the hybrid layer synergistically enhanced the hydrolytic degradation of the adhesive components and collagen fibrils, thereby weakening the physical properties of the resin–dentin bond such as bond stregth.

Specimens pretreated with NaF solution maintained its bond strength after thermocycling. Preatment of dentin surfaces with acidic solutions has been also recommended to eliminate the smear layer and enable a direct contact between the adhesive resin and dentin (34). However, main challenge for demineralizing agents is to dissolve the smear layer without demineralizing the tooth surface too profoundly (5). ITOTA *et al.* (35) showed that the bond strength of mild-self adhesive system on demineralized dentin is lower than mineralized dentin. The application of 1.23% sodium fluoride solution removes the smear layer and does not promote significant changes in the dentinal surface (6). The preservation of hydroxyapatite will also provide calcium for chemical bonding to the functional self-etch monomers (5). Within the limitations of this study, we can speculate that smear layer removal using a low pH (3.6) NaF provided better attachment between the resin monomers and dentinal components, thereby establishing a more stable hybrid layer and providing resistance to hydrolytic degradation.

Low pH of mild self-etch adhesives (pH ≈ 2) have shown to activate dentinal enzymes (36-38). Matrix metalloproteinases (MMPs) and cysteine cathepsins may be responsible for the degradation of collagen fibrils exposed at the adhesive interface (9, 10, 31, 38, 39). The use of chlorhexidine as an MMP-inhibitor was first described by HEBLING *et al*. (40) Subsequently, several authors have reported that the use of chlorhexidine associated with total-etch adhesive systems promoted the maintenance of bond strength over time (8-11). However, when chlorhexidine was used prior to the application of two-step self-etching adhesives did not maintain the resin-dentin bond strength after thermocycling (31).

For a long time, fluoride ions were used to protect hard tissues due to a favorable balance in the demineralizing and remineralizing of the enamel and dentin (41). However, recent studies have suggested that sodium fluoride has the potential to prevent collagen degradation as an MMP-inhibitor (12, 13). Purified forms of both MMP-2 and MMP-9 were completely inhibited by 200 ppm F^- (12). It is speculated that the fluoride concentration of NaF (12,300 ppm F^-) would also be able to inhibit dentinal MMPs (12). Nevertheless, little is known about the molecular mechanisms by which fluoride ions block gelatinolytic activities (12,13). Considering that MMPs are zinc and calcium-dependent, and ion F is highly electronegative, it seems logical that excess F could make these cations unavailable to participate in the catalytic

process, according to KATO *et al*. (12). The inhibition of MMPs-2 and -9 by sodium fluoride is reversible at lower F concentrations, but irreversible at higher (5,000 ppm) of F concentrations (12). This inhibitory activity of NaF associated with the the possible formation of a more homogeneous hybrid layer provided the maintenance of resin-dentin bond strengths. Thus, further studies should be conducted to confirm the potential of sodium fluoride in inhibiting dentinal MMPs, as well as its role in the preservation of collagen and maintenance of bond strength.

Within the limitations of this in vitro experimental model was observed that dentin pretreatment with acidic sodium fluoride could be useful to improve the bond stability of the bonding procedures when associated to two-step self-etch adhesive.

ACKNOWLEDGEMENTS

This work was funded thanks to grants CNPq 140058/2013-3.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. VAN MEERBEEK B, DE MUNCK J, YOSHIDA Y, INOUE S, VARGAS M, VIJAY P, VAN LANDUYT K, LAMBRECHTS P, VANHERLE G. Buonocore memorial

lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent* 2003; 28: 215-235.

2. KENSHIMA S, REIS A, UCEDA-GOMEZ N, TANCREDO LDE L, FILHO LE, NOGUEIRA FN, LOGUERCIO AD. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. *J Adhes Dent* 2005; 7: 117-126.
3. TAY F, SANO H, CARVALHO R, PASHLEY D. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. *J Adhes Dent* 2000; 2: 83-98.
4. ROCHA PI, BORGES AB, RODRIGUES JR, ARRAIS CAG, GIANNINI M. Effect of dentinal surface preparation on bond strength of self-etching adhesive systems. *Braz Oral Res* 2006; 20: 52-58
5. VAN MEERBEEK B, YOSHIHARA K, YOSHIDA Y, MINE A, DE MUNCK J, VAN LANDUYT KL. State of the art of self-etch adhesives. *Dent Mat* 2011; 27: 17-28.
6. NERI JR, PASSOS VF, VIANA FBA, RODRIGUES LKA, SABOIA VPA, SANTIAGO SL. Efficacy of smear layer removal by cavity cleaning solutions: an atomic force microscopy study. *J Dent Sci* 2011; 26: 253-257.
7. CHRISTENSEN GJ. Preventing postoperative tooth sensitivity in class I, II and V restorations. *J Am Dent Assoc* 2002; 133: 229-231.
8. BRESCHI L, CAMMELLI F, VISINTINI E, MAZZONI A, VITA F, CARRILHO M, CADENARO M, FOULGER S, MAZZOTI G, TAY FR, LENARDA R, PASHLEY D. Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: A 12-month in vitro study. *J Adhes Dent* 2009; 11: 191-198.
9. CARRILHO MRO, CARVALHO RM, DE GOES MF, DI HIPOLITO V, GERALDELI S, TAY FR, PASHLEY DH, TJÄDERHANE L. Chlorhexidine preserves dentin bond in vitro. *J Den Res* 2007; 86: 90-94.
10. LOGUERCIO AD, STANISLAWCZUK R, POLLI LG, COSTA JA, MICHEL MD, REIS A. Influence of chlorhexidine digluconate concentration and application time on resin–dentin bond strength durability. *Eur J Oral Sci* 2009; 175: 587-596.
11. SANTIAGO SL, OSORIO R, NERI JR, CARVALHO RM, TOLEDANO M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. *J Adhes Dent* 2013; 15: 535-540.
12. KATO MT, BOLANHO A, ZARELLA BL, TJÄDERHANE L, BUZALAF M. Sodium Fluoride Inhibits MMP-2 and MMP-9. *J Dent Res* 2014; 93: 74-77.

13. Mei ML, Li QL, Chu CH, Yiu CK, Lo EC. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. *Dent Mat* 2012; 28: 903-908.
14. SUYAMA Y, LÜHRS AK, DE MUNCK J, MINE A, POITEVIN A, YAMADA T, VAN MEERBEEK B, CARDOSO MV. Potential smear layer interference with bonding of self-etching adhesives to dentin. *J Adhes Dent* 2013; 15: 317-324.
15. KOIBUCHI H, YASUDA N, NAKABAYASHI N. Bonding to dentin with a self-etching primer: the effect of smear layers. *Dent Mat* 2001; 17: 122-126.
16. OGATA M, HARADA N, YAMAGUCHI S, NAKAJIMA M, PEREIRA PN, TAGAMI J. Effects of different burs on dentin bond strengths of self-etching primer bonding systems. *Oper Dent* 2001; 26: 375-382.
17. OGATA M, HARADA N, YAMAGUCHI S, NAKAJIMA M, TAGAMI J. Effect of self-etching primer vs phosphoric acid etchant on bonding to bur-prepared dentin. *Oper Dent* 2002; 27: 447-454.
18. KENSHIMA S, REIS A, UCEDA-GOMEZ N, TANCREDO LDE L, FILHO LE, NOGUEIRA FN, LOGUERCIO AD. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. *J Adhes Dent* 2005; 7: 117-126.
19. REIS A, GRANDI V, CARLOTTO L, BORTOLI G, PATZLAFF R, RODRIGUES ACCORINTE M DE L, LOGUERCIO AD. Effect of smear layer thickness and acidity of self-etching solutions on early and long-term bond strength to dentin. *J Dent* 2005; 33: 549-559.
20. TAY FR, CARVALHO R, SANO H, PASHLEY DH. Effect of smear layers on the bonding of a self-etching primer to dentin. *J Adhes Dent* 2000; 2: 99-116.
21. MINE A, DE MUNCK J, CARDOSO MV, VAN LANDUYT KL, POITEVIN A, VAN ENDE A, MATSUMOTO M, YOSHIDA Y, KUBOKI T, YATANI H, VAN MEERBEEK B. Dentin-smear remains at self-etch adhesive interface. *Dent Mater* 2014; 30:1147-1153.
22. KENSHIMA S, FRANCCI C, REIS A, LOGUERCIO AD, FILHO LE. Conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer. *J Dent* 2006; 34: 775-783.
23. ALI AA, EL DEEB HA, BADRAN O, MOBARAK EH. Bond durability of self-etch adhesive to ethanol-based chlorhexidine pretreated dentin after storage in artificial saliva and under intrapulpal pressure simulation. *Oper Dent* 2013; 38: 439-446.

24. DE CASTRO FL, DE ANDRADE MF, DUARTE JUNIOR SL, VAZ LG, AHID FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. *J Adhes Dent* 2003; 5: 129-138.
25. DALKILIC EE, ARISU HD, KIVANC BH, UCTASLI MB, OMURLU H. Effect of different disinfectant methods on the initial microtensile bond strength of a self-etch adhesive to dentin. *Lasers Med Sci* 2012; 27: 819-825.
26. MOBARAK EH. Strength durability of caries–affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. *Oper Dent* 2011; 36: 649-660.
27. SHEIKH H, HEYMANN HO, SWIFT EJ JR, ZIEMIECKI TL, RITTER AV. Effect of saliva contamination and cleansing solutions on the bond strengths of self-etch adhesives to dentin. *J Esthet Rest Dent* 2010; 22: 402-410.
28. CAMPOS EA, CORRER GM, LEONARDI DP, PIZZATTO E, MORAIS EC. Influence of chlorhexidine concentration on microtensile bond strength of contemporary adhesive systems. *Braz Oral Res* 2009; 23: 340-345.
29. ERCAN E, ERDEMIR A, ZORBA YO, ELDENIZ AU, DALLI M, INCE B, KALAYCIOGLU B. Effect of different cavity disinfectants on shear bond strength of composite resin to dentin. *J Adhes Dent* 2009; 11: 343-346.
30. SHARMA V, RAMPAL P, KUMAR S. Shear bond strength of composite resin to dentin after application of cavity disinfectants - SEM study. *Contemp Clin Dent* 2011; 2: 155-159.
31. CAMPOS EA, CORRER GM, LEONARDI DP, BARATO-FILHO F, GONZAGA CC, ZIELAK JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. *J Dent* 2009; 37: 108-114.
32. VAN LANDUYT KL, DE MUNCK J, MINE A, CARDOSO MV, PEUMANS M, VAN MEERBEEK B. Filler debonding & subhybrid-layer failures in self-etch adhesives. *J Dent Res* 2010; 89: 1045-1050.
33. SABOIA VP, SILVA FC, NATO F, MAZZONI A, CADENARO M, MAZZOTTI G, GIANNINI M, BRESCHI L. Analysis of differential artificial ageing of the adhesive interface produced by a two-step etch-and-rinse adhesive. *Eur J Oral Sci* 2009; 117: 618-624.
34. BORTOLOTTO T, FERRARI M, SUSIN A, KREJCI I. Morphology of the smear layer after the application of simplified self-etch adhesives on enamel and dentin surfaces created with different preparation methods. *Clin Oral Investig* 2009; 13: 409-417.

35. ITOTA T, TORII Y, NAKABO S, YOSHIYAMA M. Effect of fluoride application on tensile bond strength of self-etching adhesive systems to demineralized dentin. *J Prosth Dent* 2002; 88: 503-510.
36. NISHITANI Y, YOSHIYAMA M, WADGAONKAR B, BRESCHI L, MANNELLO F, MAZZONI A, CARVALHO RM, TJÄDERHANE L, TAY FR, PASHLEY DH. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. *Eur J Oral Sci* 2006; 114: 160-166.
37. OSORIO R, YAMAUTI M, OSORIO E, RUIZ-REQUENA ME, PASHLEY D, TAY F, TOLEDANO M. Effect of dentin etching and chlorhexidine application on metalloproteinase-mediated collagen degradation. *Eur J Oral Sci* 2011; 119: 79-85.
38. TOLEDANO M, YAMAUTI M, OSORIO E, OSORIO R. Zinc-inhibited MMP-mediated collagen degradation after different dentine demineralization procedures. *Caries Res* 2012; 46: 201-207.
39. TJÄDERHANE L, NASCIMENTO FD, BRESCHI L, MAZZONI A, TERSARIOL IL, GERALDELI S, TEZVERGIL-MUTLUAY A, CARRILHO MR, CARVALHO RM, TAY FR, PASHLEY DH. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. *Dent Mat* 2013; 29: 116-135.
40. HEBLING J, PASHLEY DH, TJÄDERHANE L, TAY FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. *J Dent Res* 2005; 84: 741-746.
41. TENUTA LM, CURY JA . Fluoride: its role in dentistry. *Braz Oral Res* 2010; 24(Suppl 1): 9-17.

TABLES

Table 1 – Adhesive system used, batches, chemical compositions and application protocols.

Product	Composition	Manufacturer (#Batch nº)	Application mode
Clearfil SE Bond	Primer: MDP, HEMA, water, photoinitiator Bond: MDP, BisGMA, HEMA, TEGDMA, Hydrophobics dimethacrylates, photoinitiator	Kuraray Medical, Tokyo, Japan (#51476)	Apply primer for 20 s, gently air-dry; apply bond and light cure for 10 s.

Abbreviations: BisGMA: bisphenol-A-diglycidylmethacrylate; HEMA: hydroxyethylmethacrylate; MDP: 10-methacryloyloxi-decyl-phophate; TEGDMA: triethylene-glycol-dimethacrylate.

Table 2 – Bond strengths according to the pretreatment solution. The results are in MPa ± SD (*).

Groups	Clearfil SE Bond	
	24 hours	60.000 thermocycles
Distilled water	30.98±5.96 (33) ^{A,a}	22.96±6.88 (30) ^{B,b}
CHX	30.18±4.06 (22) ^{A,a}	21.91±4.37 (26) ^{B,b}
NaF	35.60±6.52 (28) ^{A,a}	30.17±4.23 (26) ^{A,a}

Identical superscript letters indicate no statistical significance between values. Different superscript upper case letters (analysis in columns) and different superscript lower case letters (in rows) indicate statistically significant differences

(*) corresponds to the number of sticks tested per group in each period.

Table 3 – Distributions of the mode of fracture according to the study groups. Relative percentages are in ().

	Clearfil SE Bond									
	24 hours					60.000 thermocycles				
Groups	A	M	CR	CD	PF	A	M	CR	CD	PF
Distilled water	6 (16)	27 (73)	0 (0)	4 (11)	0 (0)	8 (20)	22 (55)	4 (10)	2 (5)	4 (10)
CHX	4 (14)	18 (64)	3 (11)	2 (7)	1 (4)	6 (18)	20 (61)	2 (6)	0 (0)	5 (15)
NaF	3 (8)	25 (69)	2 (6)	5 (14)	1 (3)	3 (8)	23 (66)	5 (14)	1 (4)	3 (8)

A: adhesive failure; M: mixed failure; CR: cohesive failure in resin; CD: cohesive failure in dentin; PF: premature failure

Capítulo 2

3.2 CAPÍTULO 2

Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: twelve-month results

Running title: Influence of catechin on bond strength to dentin

Authors: Jiovanne R. NERI[1,2], Monica YAMAUTI[3], Felipe D. da SILVEIRA[2], Juliano S. MENDONÇA[2], Ricardo M. de CARVALHO[4] and Sérgio L. SANTIAGO[2]

Affiliations:

[1]*Department of Restorative Dentistry, Graduate School of Dentistry, University of Fortaleza, Fortaleza, Ceará, Brazil.*

[2]*Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.*

[3] *Department of Restorative Dentistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

[4]*Department of Oral Biological and Medical Sciences, University of British Columbia, Faculty of Dentistry, Vancouver, BC, Canada.*

*Corresponding author:
Sérgio Lima Santiago
Rua Monsenhor FurtadoS/Nº
60430-355
Fortaleza, CE - Brazil
E-mail: sergiosantiago@ufc.br
Tel: +558588242704
Fax: +558533668232

ABSTRACT

Aims: To evaluate the effectiveness of dentin biomodification with epigallocatechin-3-gallate (EGCG) on the resin-dentin bonds overtime. Materials and Methods: Twenty seven extracted human third molars were prepared to expose a flat dentin surface and divided into 3 groups. Dentin surfaces were dried and treated with 20 μL aliquots of either distilled water (control); 2% chlorhexidine digluconate solution (CHX) or 0.1% EGCG aqueous solution. Solutions were rubbed for 60 s followed by bonding with Adper Easy One, and 5-mm-thick resin crown build-up. Bonded teeth were stored in distilled water for 24 h and then longitudinally sectioned to obtain bonded sticks. One-third of the specimens were immediately tested in tension at 0.5 mm/min, while the remaining specimens were tested after six and twelve months of storage in distilled water at 37°C. Data were analyzed with Two-way ANOVA and Holm-Sidak method. Results: After 24 h of storage, mean bond strength values were not significantly different among all groups ($p>0.05$). Bond strengths of EGCG and CHX remained stable after 6 and 12 months. ($p>0.05$). Conclusions: Pretreatment with EGCG or CHX preserved the bonding of Adper Easy One to dentin after six and twelve months of storage.

Keywords: Catechin; Dentin; Matrix metalloproteinases; Dental adhesive; Resin-dentin bond

INTRODUCTION

The most common cause of replacement of resin composite restorations is secondary caries.[1,2] This fact occurs due to gradual degradation of the components of the hybrid layer, such as collagen fibrils, resulting in the loss of resin-dentin bond strength and bacterial microleakage.[3,4] Promising strategies are being developed to reinforce the type I collagen and reduce their degradation, further to prevent dental caries.[5] Dentin pretreatment with bioactive agents, synthetic or natural, may produce a strong and long lasting tooth-biomaterial interface.[5-7]

Chlorhexidine has been widely used as an antimicrobial agent due to its broad spectrum of action in Gram-negative bacteria and Gram-positive, particularly *Streptococus mutans*.[8] However, there is a concern about the use of chlorhexidine as bioactive agent when adhesive procedures are employed.[9-11] Pretreatment of dentin surfaces with this agent may reduces the bond strength of self-etching adhesives to dentin.[11] Therefore, chlorhexidine should be used with caution when self-etch adhesives are selected.

Tea polyphenols have been demonstrated to be effective as antimicrobial compound against a variety of pathogenic microorganisms.[12-14] Interestingly, among the catechin family, epigallocatechin-3-gallate exhibits outstanding bactericidal efficacy due to the gallate group (gallic acid ester),[14] while other catechins do not have this effect.[15,16] Epigallocatechin-3-gallate is effective in reducing acid production in dental plaque and on the growth of *Streptococcus mutans* particularly due to suppression of specific virulence factors associated with its acidogenicity and acidurity.[17-19] Epigallocatechin-3-gallate also prevents episodes of dental demineralization, inhibiting the progress of caries in dentin.[20]

Additionally, some catechins, such as epigallocatechin-3-gallate, were able to stabilize the collagen chain.[21-23] Epigallocatechin-3-gallate increases the number of collagen fibrils crosslinks and reduces the biodegradation of collagen.[23] Recent studies showed that the use of epigallocatechin-3-gallate associated with etch-and-rinse adhesive systems was effective in preserving resin-dentin bond strength up to 6 months.[7,24] Although epigallocatechin-3-gallate is being extensively studied, their use associated with self-etch adhesives was not previously investigated.

The objective of this study was to evaluate the effectiveness of dentin biomodification with epigallocatechin-3-gallate on the resin-dentin bonds overtime. The tested null hypotheses were that 1) there will be no differences in the immediate bond strength caused by the different pretreatments; and 2) there will be no differences in bond strength between storage periods for all groups.

MATERIALS AND METHODS

Twenty seven unerupted third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the local Ethics Committee (#103/11). Selected teeth were stored in 0.1% thymol solution and used within one month after extraction. Occlusal enamel was removed using a #120-grit silicon carbide (SiC) paper mounted to an electric polishing machine (Aropol 2V; Arotec®, São Paulo, SP, Brazil) to expose a flat coronal dentin surface. The dentin surface was prepared with #600-grit SiC paper under copious water for 60 seconds to standardize the smear layer.

The teeth were randomly allocated by the Excel software (Excel 2003, Microsoft Corporatin, One Microsoft Way, Redmond, WA, USA) into 3 groups (n=9) according to the pretreatment solution prior to adhesive application. Dentin surfaces of all teeth were dried with oil/water-free air for 10 s and treated with 20 µL of either distilled water (control), 2% chlorhexidine digluconate solution (CHX) (FGM, Joinville, SC, Brazil - batch #210211) or 0.1% epigallocatechin-3-gallate aqueous solution (EGCG) (Sigma-Aldrich, Saint Louis, USA - batch #029K 1228). The solutions were rubbed on the surface with a microbrush for 60 s, and excess of each solution removed with absorbent paper.

The one-step self-etch adhesive system - Adper Easy One (3M ESPE, St. Paul, MN, USA) – was applied according to manufacturer's instructions (Table 1) and light-cured with Variable Intensity Polymerizer – VIP Junior (Bisco Inc., Schaumburg, IL, USA) with power density of 500 mW/cm^2. Five increments of 1 mm-thick of composite resin were build-up on the bonded surface (Filtek Z250; 3M ESPE, St. Paul, MN, USA - batch #1030600122) and each increment was light-cured for 20 s. The bonded teeth were stored in distilled water at 37°C for 24 h.

After storage, the bonded teeth were longitudinally sectioned in both “x” and “y” directions across the bonded interface using a diamond saw in a Labcut 1010 (Extec, Enfield, CT, USA) under water cooling to obtain bonded sticks with cross-sectional area of approximately 1.0 mm^2. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm and recorded for subsequent calculation of bond strength values.

Sticks from each tooth were randomly allocated by the Excel software (Excel 2003, Microsoft Corporatin, One Microsoft Way, Redmond, WA, USA) and assigned to three storage times: 24 hours, 6 months and 12 months. All sticks were stored in distilled water at 37°C and the storage solution was changed every two weeks. For microtensile test, each bonded stick was

attached with cyanoacrylate glue (Super Bonder Gel, Loctite, São Paulo, SP, Brazil) to a modified Geraldeli testing apparatus (Odeme Biotechnology, Joaçaba, SC, Brazil) and subjected to a tensile force at 0.5 mm/min in a universal testing machine (Instron 3345; Instron Inc., Canton, MA, USA). The load at fracture was used to calculate bond strength (MPa).

The failure mode was evaluated using a stereoscope at 80X magnification (StereoZoom® Leica S8 APO, Leica Microsystems, Wetzlar, Germany), and classified as cohesive when fracture occurred exclusively within dentin (CD) or resin composite (CR); adhesive (A) when was at the dentin/resin interface, or mixed (M) when two modes of failure (adhesive and cohesive) occurred simultaneously.

Statistical procedures were performed with the Sigmastat 3.5 (Systat Software Inc., San Jose, CA, USA*)* for Windows statistical program software. A Shapiro-Wilk test was applied to all groups to analyze the normal distribution of errors and the Barlett test for the homoscedasticity. Bond strength values were statistically analyzed with Two-way ANOVA (pretreatment and storage) and Holm-Sidak method was used for *post hoc* comparisons. Statistical significance was set at $p<0.05$. Teeth were used as a statistical unit and the number of prematurely debonded specimens was recorded, although this was not included in the analysis.

RESULTS

The results were affected by treatment ($p<0.001$; $F=17.562$), but not by storage time ($p=0.295$; $F=1.242$). Interactions were statistically significant ($p=0.01$; $F=3.573$) (Table 2).

After 24 h storage, there was no significant difference among the bond strength values of all groups ($p>0.05$). At 6 months and 12 months, bond strength mean values of EGCG, and CHX groups were not statistically different ($p>0.05$) and they were significantly higher than those of distilled water (control, $p<0.05$). In addition, the resin-dentin bond strength was preserved up to 12 months after application of EGCG and CHX ($p>0.05$)

Most of failures were mixed in all tested groups at all storage times. At 24 h, adhesive failures were more observed than cohesive failures (resin and dentin) for specimens of CHX group. The number of adhesive failures increased with ageing, except in CHX group (Table 3).

DISCUSSION

Dentin pretreatment with EGCG or CHX did not affect immediate bond strength as the control group; therefore failed to reject the first hull hypothesis . On the other hand, the second null hypothesis was rejected since that only experimental solutions used as pretreatment were effective in preserving the bond strength after 6 and 12 months of water storage.

The use of chlorhexidine as bioactive agent prior to application of self-etch adhesives is controversial. Some studies[9-11,25] suggested that such use should be avoided because there could be interactions between chlorhexidine and the adhesive components. These interactions could decrease their wettability and the level of dentin conditioning.[9] However, the results of the present study demonstrated that chlorhexidine application prior to bonding procedures did not present adverse effects on immediate resin-dentin bond strength for self-etch adhesive (Table 2) and this data is confirmed by other studies.[26-30]

Dentin pretreatment with epigallocatechin-3-gallate has also been shown not to be detrimental to bond strengths to dentin.[7,24] In the study published by Du et al.[24] there were no difference between degrees of conversion among concentrations of epigallocatechin-3-gallate used, although these authors speculated that higher concentration of epigallocatechin-3-gallate, e.g., 0.03% could disturb the polymerization of the adhesive, thus affecting bond strength. However, EGCG at 0.1% was used in the present study and did not significantly affect bond strength (Table 2). Neri et al.[31] demonstrated that incorporation of epigalocatechin-3-gallate at 0.1% in Adper Easy One did not present adverse effects on degree of conversion. Probably, the EGCG at 0.1% have not been entrapped within the linear chains after curing without interfering with monomers conversion and bond strength.

The mode of failure of the tested groups confirmed the bond strength results at 24 h, as the control, CHX and EGCG groups showed almost similar results, with an increased percentage of mixed failures (Table 3). The results of the control group corroborated with others studies,[30,32] which showed high rates of mixed failure with Adper Easy One. This was explained by the micromechanical as well as chemical interaction of the functional monomer included in this adhesive with the hydroxyapatite crystals that remain at the surface.[32]

Enzymatic degradation of the collagen matrix by host derived enzymes plays a significant role in the destruction of the bonded interface.[33] Matrix metalloproteinases (MMPs) and cysteine cathepsins have been identified in dentin in inactive form.[34,35] However, some researches have reported that the low pH of self-etch adhesive systems (pH 2.3-5) could activate

dentin enzymes.[34,36,37] The self-etch adhesive used in this study has a pH value of 2.4 and so, is capable of enhancing dentin proteolytic activity without denaturation of the enzymes. It has been suggested that the action of these enzymes may be responsible for the degradation of collagen fibrils exposed at the adhesive interface.[6,34,35] After 6 and 12-month storage in distilled water at 37 °C, it was observed the reduction of the bond strength values for the control group (Table 2). These data corroborate the findings of other *in vitro* studies.[28,30,38] Probably, the action of MMPs and cysteine cathepsins has determined to reduce the bond strength of the control group as well as the increase of number of adhesive and premature (Table 3).

On the other hand, the use of bioactive substances that inhibit the enzymes may help to preserve the hybrid layer and increase the durability of resin-dentin bonded interfaces.[4,6,7]. Besides being an excellent antimicrobial agent, chlorhexidine is also an inhibitor of the activity of MMP-2, MMP-8 and MMP-9.[39] The use of chlorhexidine improved the integrity of the collagen network within hybrid layer after aging.[40-42] When chlorhexidine was applied as a pretreatment resulted in the maintenance of bond strength over 6 months[30] and 12 months, corroborating with our results.

As chlorhexidine, epigallocatechin-3-gallate has been used as dentin pretreatment associated with etch-and-rinse adhesive systems and it was effective in preserving bond strength up to 6 months.[7,24] According to the results of the present study, epigallocatechin-3-gallate seems to be able to maintain the stability of resin-dentin bond for up to 12 months (Table 2). One reason for maintaining the bond strength over time by epigalocatechin-3-gallate may be due of its ability to enhance of collagen biomechanical properties.[23,43] Collagen stabilization depends directly of the specific chemical structures of the bioactive agent involved in the process.[5,43] Thus, epigalocatechin-3-gallate molecule containing galloyl groups can stabilize dentin collagen through hydrogen bonding and hydrophobic interactions.[21,22,43] The greater ability by epigalocatechin-3-gallate to form colagen cross-links increases the ultimate tensile strength and elastic modulus of demineralized dentin.[43]

Another plausible reason to improve the durability of the resin-dentin bond strength, is the fact of epigalocatechin-3-gallate inhibits of collagen-degrading enzymes action, such as MMP[43,44] and cysteine cathepsins[43,44]. The mechanism of action of epigalocatechin-3-gallate is likely more complex, involving multi-functional activities, such as down regulation of endogenous proteases expression,[44] protease inactivation/silencing[43-44] and preventing the free access of collagenase to active sites on the collagen chains[23,43]. The limitation of this study is the lack of evidence of inhibition enzyme activity by EGCG within the clinical application parameters used. Although epigalocatechin-3-gallate present promising results as dentin

pretreatment, it is necessary simplify the protocol of application. Thus, further studies should incorporate this catechin directly into the self-etch adhesive systems and analyze its effect on physicochemical properties.

CONCLUSIONS

Epigallocatechin-3-gallate and chlorexidine when used as bioactive agent before Adper Easy One application did not affect the immediate resin-dentin bond strength and preserved the resin-dentin bond strength up to twelve months.

ACKNOWLEDGEMENTS

This work was funded thanks to grants CNPq 140058/2013-3.

REFERENCES

1. Kidd EA Caries diagnosis within restored teeth. Adv Dent Res. 1990;4:10-13.
2. Kopperud SE, Tveit AB, Gaarden T, Sandvik L, Espelid I. Longevity of posterior dental restorations and reasons for failure. Eur J Oral Sci. 2012;120:539-548.
3. Hebling J, Pashley DH, Tjäderhane L, Tay FR Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2005;84:741-746.
4. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008;24:90-101.
5. Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, Aguiar TR, Vidal CM, Napotilano JG, Nam JW, Leme AA. Dentin biomodification: strategies, renewable resources and clinical applications. Dent Mater 2014;30:62-76.
6. Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, Reis A. Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. Eur J Oral Sci 2009;117:587-596.
7. Santiago SL, Osorio R, Neri JR, Carvalho RM, Toledano M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. J Adhes Dent 2013;15:535-540.
8. Christensen GJ. Preventing postoperative tooth sensitivity in class I, II and V restorations. J Am Dent Assoc 2002;133:229-231.
9. Campos EA, Correr GM, Leonardi DP, Pizzatto E, Morais EC. Influence of chlorhexidine concentration on microtensile bond strength of contemporary adhesive systems. Braz Oral Res 2009;23:340-345.
10. Ercan E, Erdemir A, Zorba YO, Eldeniz AU, Dalli M, Ince B, Kalaycioglu B. Effect of different cavity disinfectants on shear bond strength of composite resin to dentin. J Adhes Dent. 2009;11:343-346.
11. Sharma V, Rampal P, Kumar S. Shear bond strength of composite resin to dentin after application of cavity disinfectants - SEM study. Contemp Clin Dent 2011;2:155-159.
12. Yoda Y, Hu ZQ, Zhao WH, Shimamura T. Different susceptibilities of Staphylococcus and Gram-negative rods to epigallocatechin gallate. J Infect Chemother 2004;10:55–58.

13. Maeyama R, Kwon IK, Mizunoe Y, Anderson JM, Tanaka M, Matsuda T. Novel bactericidal surface: Catechin-loaded surface-erodible polymer prevents biofilm formation. J Biomed Mater Res A 2005;75:146-155.
14. Lee P, Tan KS. Effects of Epigallocatechin gallate against Enterococcus faecalis biofilm and virulence. Arch Oral Biol 2015;60:393-399.
15. Amarowicz R, Pegg RB, Bautista DA. Antibacterial activity of green tea polyphenols against Escherichia coli K 12. Nahrung 2000;44:60–62.
16. Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 2004;11:380– 381.
17. Hirasawa M, Takada K, Otake S. Inhibition of acid production in dental plaque bacteria by green tea catechins. Caries Res 2006;40:265-270.
18. Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of Streptococcus mutans. Antimicrob Agents Chemother 2011;55:1229–1236.
19. Xu X, Zhou XD, Wu CD. Tea catechin epigallocatechin gallate inhibits Streptococcus mutans biofilm formation by suppressing gtf genes. Arch Oral Biol. 2012;57:678-683.
20. Chen HQ, Huang B. Effect of EGCG Application on Collagen Degradation in Dentine Caries. Appl Mech Mater 2014;455:112-116.
21. Goo HC, Hwang YS, Choi YR, Cho HN, Suh H. Development of collagenase-resistant collagen and its interaction with adult human dermal fibroblasts. Biomaterials 2003;24:5099–5113.
22. Tang HR, Covington AD, Hancock RA. Structure-activity relationships in the hydrophobic interactions of polyphenols with cellulose and collagen. Biopolymers. 2003;70:403–413.
23. Jackson JK, Zhao J, Wong W, Burt HM. The inhibition of collagenase induced degradation of collagen by the galloyl-containing polyphenols tannic acid, epigallocatechin gallate and epicatechin gallate. J Mater Sci Mater Med 2010;21:1435-1443.
24. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. Journal of Dentistry 2012;40:485-492
25. Shafiei F, Alikhani A, Alavi AA. Effect of chlorhexidine on bonding durability of two self-etching adhesives with and without antibacterial agent to dentin. Dent Res J 2013;10:795-801.

26. de Castro FL, de Andrade MF, Duarte Junior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. J Adhes Dent 2003;5:129-138.
27. Sheikh H, Heymann HO, Swift EJ Jr, Ziemiecki TL, Ritter AV. Effect of saliva contamination and cleansing solutions on the bond strengths of self-etch adhesives to dentin. J Esthet Restor Dent 2010;22:402-410.
28. Mobarak EH. Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. Oper Dent 2011;36:649-660.
29. Dalkilic EE, Arisu HD, Kivanc BH, Uctasli MB, Omurlu H. Effect of different disinfectant methods on the initial microtensile bond strength of a self-etch adhesive to dentin. Lasers Med Sci 2012;27:819-825.
30. Ali AA, El Deeb HA, Badran O, Mobarak EH. Bond durability of self-etch adhesive to ethanol-based chlorhexidine pretreated dentin after storage in artificial saliva and under intrapulpal pressure simulation. Oper Dent 2013;38:439-446.
31. Neri JR, Yamauti M, Feitosa VP, Pires APM, Araújo RS, Santiago SL. Physicochemical properties of a methacrylate-based dental adhesive incorporated with epigallocatechin-3-gallate. Braz Dent J 2014;25:528-531.
32. Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Kuboki T, Yoshida Y, Suzuki K, Lambrechts P, Van Meerbeek B. Bonding effectiveness of two contemporary self-etch adhesives to enamel and dentin. J Dent 2009; 37:872-883.
33. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216-221.
34. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res 2011;90:953-968.
35. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-135.
36. Nishitani Y, Yoshiyama M, Wadgaonkara B, Breschi L, Mannelo F, Mazzoni A, Carvalho RM, Tjäderhane L, Tay FR, Pashley DH. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci 2006;114:160–166.

37. Toledano M, Yamauti M, Osorio E, Osorio R. Zinc-inhibited MMP-mediated collagen degradation after different dentine demineralization procedures. Caries Res 2012;46:201-207.
38. Campos EA, Correr GM, Leonardi DP, Barato-Filho F, Gonzaga CC, Zielak JC. Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing. J Dent 2009;37:108-114.
39. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol 1999;6:437-439.
40. Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2005;84:741-746.
41. Sanabe ME, Costa CA, Hebling J. Exposed collagen in aged resin-dentin bonds produced on sound and caries-affected dentin in the presence of chlorhexidine. J Adhes Dent 2011;13:117-124.
42. Lafuente D. SEM analysis of hybrid layer and bonding interface after chlorhexidine use. Oper Dent 2012;37:172-180.
43. Vidal CM, Aguiar TR, Phansalkar R, McAlpine JB, Napolitano JG, Chen SN, Araújo LS, Pauli GF, Bedran-Russo A. Galloyl moieties enhance the dentin biomodification potential of plant-derived catechins. Acta Biomater 2014;10:3288-3294.
44. Garbisa S, Sartor L, Biggin, S. Salvato, B, Benelli, R, Albini A. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer 2001;91:822-832.
45. Devika PT, Stanely Mainzen Prince P. (-)Epigallocatechin-gallate (EGCG) prevents mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats: a transmission electron microscopic and in vitro study. Pharmacol Res 2008;57:351-357.

TABLES

Table 1 – Adhesive system and bonding procedure.

Product	Composition	Manufacturer (#Batch nº)	Application mode
Adper Easy One*	Adhesive–Bis-GMA, HEMA, Methacrylated phosphoric esters dimethacrylates, 1,6 hexanediol dimethacrylate, polyalquenoic acid copolymer, silica nanofiller (7 nm), initiators, water and ethanol	3M ESPE, St.Paul, MN, USA (#402261)	1-apply one coat of adhesive for 20 s; 2-air-drying for 5 s; 3-light-curing for 10 s.

Abreviations: Bis-GMA: bisphenol A diglycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate. *This brand name is the same product as Adper Easy Bond.

Table 2 – Bond strength values (MPa ± SD (*)) according to pretreatment solution.

Groups	Adper Easy One		
	24 hours	6 months	12 months
Distilled water	23.5±4.3 (32) ^{A,a}	16.7±6.1 (30) ^{B,b}	13.8±4.5 (37) ^{B,b}
CHX	24.5±6.6 (37) ^{A,a}	26.5±5.9 (37) ^{A,a}	25.2±5.2 (43) ^{A,a}
EGCG	24.7±4.4 (32) ^{A,a}	25.4±5.5 (33) ^{A,a}	26.8±5.0 (40) ^{A,a}

Identical superscript letters indicate no statistical significance between values. Capital letters compare treatments and lower cases compare storage time.

(*) corresponds to the number of sticks tested per group in each period

Table 3 – Distribution of mode of fracture of each group expressed as *n* (relative percentage)

Groups	Adper Easy One														
	24 hours					6 months					12 months				
	A	M	CR	CD	PF	A	M	CR	CD	PF	A	M	CR	CD	PF
Distilled water	3 (8)	29 (76)	3 (8)	0 (0)	3 (8)	2 (5)	28 (74)	2 (5)	1 (3)	5 (13)	11 (27)	25 (63)	1 (3)	0 (0)	3 (7)
CHX 2%	12 (27)	25 (55)	5 (11)	0 (0)	3 (7)	10 (23)	27 (61)	4 (9)	0 (0)	3 (7)	10 (21)	32 (68)	4 (9)	0 (0)	1 (2)
EGCG 0.1%	2 (5)	30 (77)	6 (15)	0 (0)	1 (3)	6 (18)	27 (79)	0 (0)	0 (0)	1 (3)	8 (19)	31 (74)	2 (5)	0 (0)	1 (2)

A: adhesive failure; M: mixed failure; CR: cohesive failure in resin; CD: cohesive failure in dentin; PF: premature failure

Capítulo 3

3.3 CAPÍTULO 3

Physicochemical properties of a methacrylate-based dental adhesive incorporated with epigallocatechin-3-gallate

Running title: Properties of adhesive doped with catechin

Authors: Jiovanne Rabelo NERI[1,2], Monica YAMAUTI[2], Victor Pinheiro FEITOSA[2], Amanda Pontes Maia PIRES[3], Rinaldo dos Santos ARAÚJO[3] and Sérgio Lima SANTIAGO[2]

Affiliations:

[1]*Department of Restorative Dentistry, Graduate School of Dentistry, University of Fortaleza, Fortaleza, Ceará, Brazil.*

[2]*Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.*

[3]*Department of Chemistry and Environmental Technology, Federal Institute for Education, Science and Technology of Ceará, Fortaleza, Ceará, Brazil.*

*Corresponding author:

Dr. Sérgio Lima Santiago

E-mail: sergiosantiago@ufc.br

Tel: +558588242704

Fax: +558533668232

ABSTRACT

This study aimed to evaluate the influence of epigallocatechin-3-gallate (EGCG) incorporation on the physicochemical properties of a methacrylate-based dental adhesive. EGCG was added to Adper Easy One (3M-ESPE), except in control group, to obtain concentrations of 0.01% w/w and 0.1% w/w of EGCG-doped adhesives. For water sorption (WS) and solubility (SL) surveys, resin discs were assayed following ISO recommendations (n=10). The degree of conversion (DC) was analyzed by FTIR whereas flexural strength (FS) was tested in three-point bending with bar specimens (n=10). Data were subjected to one-way ANOVA and Tukey's test ($p<0.05$). No significant difference in the DC, WS and FS were found between the different concentrations of EGCG ($p>0.05$). Adhesives containing 0.1% or 0.01% of EGCC demonstrated similar values of SL ($p>0.05$); and lower than those found for adhesive without EGCC ($p<0.05$). In conclusion, the addition of EGCC to adhesive reduced the solubility without affect the other properties evaluated.

Keywords: Mechanical phenomena; Dental adhesives; Flavonoids.

INTRODUCTION

Dental adhesives have created several alternatives for direct build-ups and other restorative treatments since their introduction more than forty years ago (1). However, their bonding performance on dentin may not be as durable as previously assumed (2). The resin-dentin bonds attained by using most of the contemporary adhesives can deteriorate over time (3,4).

A recent review has indicated that the hybrid layer created by the *in situ* polymerization of resin monomers (generally dimethacrylates) infiltrated in a partially demineralized dentin organic matrix is the weak link in the adhesive restorations (5). Aging may affect most of the components in the resin-dentin interface individually or several simultaneously (6). The dentin bond degradation may occur via two mechanisms: (1) the hydrolytic degradation of the polymer within the hybrid and adhesive layers and (2) the breakdown of the resin-sparse collagen fibrils (1,2). By self-etching the dental substrates without a separate application of phosphoric acid, the zone of resin-sparse collagen is reduced (6).

Overall, the self-etch adhesives contain relatively more hydrophilic components since acidic functional monomers and water are needed to a feasible etching of dentin and enamel (7,8). Therefore, these more hydrophilic adhesives tend to rapidly absorb water (7).Water transudation accelerates the swelling, plasticization and the elution of unreacted monomers (9). Furthermore, the accelerated degradation of the collagen by host-derived enzymes (i.e. matrix-metalloproteinases and cathepsins) plays a significant role on the degradation of dentin bonds (10). Thus, both mechanisms could lead to degradation of the dentin bond (7,10) as well as the drop on the physical properties of dental adhesives (11) by the polymer hydrolysis.

Some natural chemicals, such as flavonoids (present in fruits, vegetables, nuts, seeds and flowers) have shown enzymatic inhibition (4) and potential collagen cross-linking (12). Epigallocatechin-3-gallate (EGCG) is the principal flavonoid of green tea (*Camelia sinesis*), and it has been investigated due to its potential against cancer (13). Notably, this polyphenol has been demonstrated to be an effective inhibitor of matrix-metalloproteinases (MMPs) and cysteine cathepsins (13). It has been shown that EGCG can increase the collagen crosslinking and prevent the free access of collagenase to the active sites on the collagen chains (14). Recent studies demonstrated the beneficial effects of epigallocatechin-3-gallate mixed in dental adhesive which maintained resin-dentin bond strength over time (3,4). Nevertheless, the effects of this mixture on the physicochemical properties of self-etch dental adhesives have never been previously evaluated.

Thus, the aim of this study was to evaluate the influence of epigallocatechin-3-gallate incorporation on the physicochemical properties of a commercial methacrylate-based dental bonding agent. The hypothesis to be tested was that different concentrations of EGCG do not cause a detrimental effect on the water sorption/solubility, the degree of conversion and the flexural strength of the adhesive.

MATERIAL and METHODS

1. Doping the adhesive with EGCG

A commercial one-step self-etch adhesive, Adper Easy One (EO, also known as Adper Easy Bond, 3M ESPE, St. Paul, MN, USA – batch #402261), was used in this study. Epigallocatechin-3-gallate (EGCG, Sigma Aldrich, St. Louis, MO, USA) was added to the adhesive system in 0.01% w/w (EGCG 0.01) or 0.1% w/w (EGCG 0.1) concentrations. In order to obtain a homogenous mixture, the EGCG-doped adhesives were shaken using a tube agitator (QL-901,Biomixer, São Paulo, SP, Brazil) in darkness for 1 min. The homogeneity of the dilution was carefully checked and it was used only if no crystals were noted. The three tested adhesive systems (EO - Control, EGCG 0.01 and EGCG 0.1) were evaluated *in vitro* using water sorption, water solubility, degree of conversion and flexural strength experiments.

2. Water sorption/solubility

Water sorption and solubility were determined following ISO 4049:2000 except for specimen dimensions as previously undertaken by Ito et al.(15).

The disc specimens (n=10) were prepared by using a Teflon matrix (10 mm diameter and 1 mm thickness). A Mylar strip and a glass slide were placed on the discs after dispensing the adhesive systems using micro-pipettes. The light-activation was undertaken for 40 s using a halogen lamp light source (VIP junior, Bisco Inc., Schaumburg, IL, USA) with 500 mW/cm² irradiance at both sides of each specimen and they were ground and slowly polished up to a thickness of 0.5 mm using 600-grit SiC polishing papers.

The discs were stored in a silica-containing desiccator at 37°C and were repeatedly weighed after a 24 h intervals on an analytical balance (AUX-220, Shimadzu, Tokyo, Japan) with an accuracy of 0.0001 g up to a constant mass (m1) was obtained (i.e.,variation less than 0.1 mg in three weight measures). The volume of each specimen were measured with a 0.001 mm precision digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) by analyzing the diameter and thickness, and the volume (V) was expressed in mm^3. Thereafter, the specimens were stored in sealed glass vials with 10 ml of distilled water at 37°C for 7 days. Afterwards, the specimens were weighed after gently wiped on absorbent papers to obtain a constant mass (m2) and then they were returned to the desiccator. The specimens were finally weighed as aforementioned up to stabilization of a constant mass (m3). Water sorption (WS) and solubility (SL) ($\mu g/mm^3$) were calculated using the following formulae:

$$WS = \frac{m2 - m3}{V} \qquad SL = \frac{m1 - m3}{V}$$

The data were statistically analyzed using one-way ANOVA and Tukey's test ($p<0.05$).

3. Degree of conversion

The degree of conversion (DC) of the adhesive resins was assessed by Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer Spectrum 100, Perkin Elmer, Shelton, USA). Each adhesive system was dispensed into a small agate mortar and thoroughly mixed with potassium bromide (KBr) using a pestle, at a ratio of 4:100 w/w. The pellets of KBr/adhesive solution were prepared with a hand press (Hand Press Kit 161-1100, PIKE Technologies, Madison, WI, USA). FTIR spectrum of the uncured adhesive was obtained from each sample using 32 scans in a range of 4000-400 cm^{-1}, at 4 cm^{-1} resolution in transmission mode.

The adhesive resins were light-activated for 20 s using the light source (VIP junior, Bisco Inc., Schaumburg, IL, USA). Additional FTIR spectra were obtained immediately after light curing. The analyses were performed at room temperature with 50% relative humidity. Ten specimens per group (n=10) were tested. The rate of unreacted carbon-carbon double bonds (C=C) was determined from the ratio of absorbance intensities of aliphatic C=C (peak at 1636 cm^{-1}) against an internal standard (aromatic carbon-carbon bond peak at 1608 cm^{-1}) before and after curing. Degree of conversion was determined by subtracting the C=C from 100%. Data were statistically analyzed using one-way ANOVA and Tukey's test ($p<0.05$).

4. Flexural Strength

A three-point bending test was used to assess the flexural strength (FS) of bar-shaped specimens. Twenty microliters of each adhesive resin was placed into a Teflon matrix to prepare the bar-shaped specimens (25 mm length x 2 mm width x 2 mm height) according to ISO 4049:2000. The adhesive resins were following air-dried for 20 s for solvent evaporation. Before the light-activation, a Mylar strip was placed on the top of the matrix and covered with a glass slide.

The adhesives were light-activated for 40 s with the light source (VIP junior, Bisco Inc., Schaumburg, IL, USA) at 3 different positions (right, middle and left) of sample. After light activation, the specimens were removed from the matrix, and the bottom surface underwent additional light-activation for 40 s. Specimens were stored for 24 h in distilled water at 37°C and subjected to a three-point bending test using a universal testing machine (Instron 3345, Instron Corp., Canton, MA, USA) at a crosshead speed of 1.0 mm/min. The dimensions of each specimen were captured using a digital caliper (0.01 mm precision, Absolute Digimatic, Mitutoyo, Tokyo, Japan). The FS calculated and transformed to MPa according to the formula:

$$FS = \frac{3FI}{2wb^2}$$

F=maximum force (N) at the fracture; I=the distance between the supports (fixed at 20 mm), w=specimen width; and b=specimen thickness.

Data were statistically analyzed using one-way ANOVA and Tukey's test ($p<0.05$).

RESULTS

The results of the study are presented in Table 1. There was no significant difference in the WS between all groups ($p=0.158$). On the other hand, the SL outcomes were significantly different ($p<0.001$) presenting reduced solubility by increasing the EGCG concentration.

The degree of conversion was significantly similar between all tested adhesive resins ($p=0.214$). There were no significant differences in the FS values between all groups ($p=0.313$).

DISCUSSION

The main active component of green tea is epigallocatechin-3-gallate (EGCG). It has been shown several outstanding therapeutic effects on oral health (16). Two principal properties of EGCG related to restorative/conservative dentistry are its bacteriostatic activity (17) on Streptococcus mutans, one of the main bacteria responsible for the progress of dental caries, and its MMP inhibition potential which strikingly prevents dentin collagen degradation (18). Furthermore, dentin treatment using EGCG significantly improved the mechanical properties of demineralized dentin which suggests potential collagen crosslinking (19). Previous investigations also demonstrated the action of EGCG in very low concentrations (3,17). Therefore, the use of very low concentrations of EGCG as used in the present study might be suitable to attain its potential therapeutic effects.

The water sorption of dental polymers may be positively correlated with the hydrophilicity of the overall components (7-9,15). Most dental methacrylate-based monomers as well as photoinitiators contain polar functionalities such as esters and hydroxyls (20). The polarity of adhesive systems influences their affinity with water molecules (8).Though epigallocatechin-3-gallate is a relatively polar molecule due to several hydroxyls (21,22), no increase in the water sorption was attained in EGCG-doped adhesives (Table 1). Pallan et al. showed that the water sorption of different resins was associated with their formulations (hydrophilic components/features) instead of the presence of epigallocatechin-3-gallate (23). Indeed, the low concentrations of EGCG (0.01% and 0.1%) in the present investigation were not able to cause a significant increase in the overall hydrophilicity of the one-step self-etch adhesive, which could result insignificantly increased water sorption.

Simplified adhesives (i.e. one-step self-etch adhesives) often exhibit relatively high water sorption and solubility (9,15). High solubility was also found when epigallocatechin-3-gallate (1 wt% and 2 wt%) was incorporated to adhesive resins (23). It was speculated that the increase in solubility of EGCG-doped resins occurs due to the release of EGCG along with residual monomers and oligomers. Contrariwise, in the present study, the incorporation of EGCG (0.01% and 0.1%) reduced the SL of the tested resin. Such outcome may be explained by the difficult achievement of a homogeneous mixture between the branched catechin (EGCG) molecule and the polymer network. Potential hydrogen bonds between the EGCG hydroxyls, HEMA and Bis-GMA (23) are also feasible linkages which could impair the releasing of components resulting in lower solubility.

The degree of conversion is a useful strategy to assess the basic physicochemical properties of a dental resin, such as maximum polymerization reaction (24). The present findings demonstrated that the incorporation of EGCG in low concentrations into a specific one-step self-etch adhesive did not cause any negative effect on the DC (Table 1). These results are corroborated by recent studies (3,23). Du et al. speculated that high EGCG concentrations (more than 0.03%) could induce the formation of linear polymer chains, and the free radical scavenging effect of EGCG may jeopardize the polymerization (3). Indeed, the low concentrations of EGCG may have avoided these phenomena without interfering with polymerization conversion.

The final conversion after light-curing affects several mechanical properties such as the flexural strength (24). Nevertheless, polymers with similar degree of conversion may exhibit different crosslink densities which may afford contrasting flexural strengths (25). Once EGCG incorporation did not affect the FS (Table 1), we may assume that the crosslinking of the tested resins is similar. In fact, the same crosslinking of the polymer matrix play an important role on the resemblance in the water sorption among all concentrations of EGCG.

The observed results showed that the incorporation of EGCG into Adper Easy One, a one-step self-etch adhesive, did not affect its physicochemical properties, except the solubility. This leads to a partial acceptance of the hypothesis. A limitation of this study was not quantifying the release of EGCG from the adhesive system. Therefore, further studies are encouraged to assess the releasing of EGCG in order to inhibit proteinases and improve the resin-dentin bonds over time. In conclusion, EGCG incorporation (0.01% and 0.1%) into a self-etch dental adhesive may be useful for therapeutic adhesion to dental hard substrates, particularly to dentin, due to the potential of improving the longevity of adhesive procedures.

RESUMO

O presente estudo teve como objetivo avaliar a influência da incorporação de epigalocatequina-3-galato (EGCG) nas propriedades físico-mecânicas de um sistema adesivo. O EGCG foi adicionado ao Adper Easy One (3M-ESPE), exceto para o grupo controle, para a obtenção das concentrações de 0,01% e 0,1% p/p. No ensaio de sorção (S) e solubilidade (SL), foram confeccionados discos de resina de acordo com as recomendações da ISO (*n*=10). O grau de conversão (GC) foi analisado através de FTIR, enquanto a resistência flexural (RF) foi avaliada em um teste de flexão em três pontos com espécimes em forma de barra (n=10). Os dados foram submetidos à Análise de Variância a um critério e teste de Tukey ($p<0.05$). Não houve diferença significativa entre as concentrações de EGCG testadas no GC, S e RF ($p>0,05$). Adesivos contendo EGCG a 0,1% ou 0,01% apresentaram valores similares de SL ($p>0,05$); e inferiores aos valores obtidos pelo adesivo não incorporado por EGCG. Conclui-se que a adição de EGCG ao adesivo reduziu a solubilidade sem afetar as outras propriedades avaliadas.

REFERENCES

1. Breschi L, Cammelli F, Visintini E, Mazzoni A, Vita F, Carrilho M, Cadenaro M, Foulger S, Mazzoti G, Tay FR, Lenarda R, Pashley D. Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: A 12-month in vitro study. J Adhes Dent 2009; 11: 191-198.
2. Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidin arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res 2005; 84: 741-746.
3. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent 2012; 40: 485-492.
4. Santiago SL, Osorio R, Neri JR, Carvalho RM, Toledano M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. J Adhes Dent 2013; 15: 535-540.
5. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, Eslick J, Camarda K, Katz JL. Adhesive/dentin interface: the weak link in the composite restoration. Ann Biomed Eng 2010; 38: 1989-2003.
6. Breschi L, Mazzoni A, Rugger A, Cadenaro M, di Lenarda R, de Stefano Dorigo E. Dental adhesion review: Aging and stability of the bonded interface. Dent Mater 2008; 24: 90-101.
7. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, Carrilho MR. Water sorption/solubility of dental adhesive resins. Dent Mater 2006; 22: 973-980.
8. Yiu CK, King NM, Carrilho MR, Sauro S, Rueggeberg FA, Prati C, Carvalho RM, Pashley DH, Tay FR. Effect of resin hydrophilicity and temperature on water sorption of dental adhesive resins. Biomaterials 2006; 27: 1695-1703.
9. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005; 26: 6449-6459.
10. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins.Dent Mater 2013; 29: 116-135.

11. Hosaka K, Tagami J, Nishitani Y, Yoshiyama M, Carrilho M, Tay FR, Agee KA, Pashley DH. Effect of wet vs. dry testing on the mechanical properties of hydrophilic selfetching primer. Eur J Oral Sci 2007; 115: 239-245.
12. Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. J Biomed Mater Res B ApplBiomater 2007; 80: 268-272.
13. Garbisa S, Sartor L, Biggin, S. Salvato, B, Benelli, R, Albini A. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer 2001; 91: 822-832.
14. Jackson JK, Zhao J, Wong W, Burt HM. The inhibition of collagenase induced degradation of collagen by the galloyl-containing polyphenols tannic acid, epigallocatechingallate and epicatechingallate. J Mater Sci Mater Med 2010; 21: 1435-1443.
15. Ito S, Hoshino T, Iijima M, Tsukamoto N, Pashley DH, Saito T. Water sorption/solubility of self-etching dentin bonding agents. Dent Mater 2010; 26: 617-626.
16. Narotzki B, Reznick AZ, Aizenbud D, Levy Y. Green tea: a promising natural product in oral health. Arch Oral Biol 2012; 57: 429-435.
17. Mankovskaia A, Lévesque CM, Prakki A. Catechin-incorporated dental copolymers inhibit growth of Streptococcus mutans. J Appl Oral Sci 2013; 21: 203-207.
18. Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhães AC, Pereira JC, Buzalaf MA. Impact of protease inhibitors on dentin matrix degradation by collagenase. J Dent Res 2012; 91: 1119-1123.
19. Hiraishi, N., Sono, R., Sofiqul, I., Yiu, C., Nakamura, H., Otsuki, M., Takatsuka, T., Tagami J. In vitro evaluation of plant-derived agents to preserve dentin collagen. Dent Mater 2013; 29: 1048-1054.
20. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater 2006; 22: 211-222.
21. Kim WJ, Kim JD, Kim J, Oh SG, Lee YW. Selective caffeine removal from green tea using supercritical carbon dioxide extraction. J Food Eng 2008; 89: 303-309.
22. Lambert JD, Sang S, Hong J, Kwon SJ, Lee MJ, Ho CT, Yang CS. Peracetylation as a means of enhancing in vitro bioactivity and bioavailability of epigallocatechin-3-gallate. Drug MetabDispos 2006; 34: 2111-2116.

23. Pallan S, Furtado Araujo MV, Cilli R, Prakki A. Mechanical properties and characteristics of developmental copolymers incorporating catechin or chlorhexidine. Dent Mater 2012; 28: 687-694.
24. Arrais CA, Pontes FM, Santos LP, Leite ER, Giannini M. Degree of conversion of adhesive systems light-cured by LED and halogen light. Braz Dent J 2007;18:54-59.
25. Ferracane JL. Correlation between hardness and degree of conversion during the setting reaction of unfilled dental restorative resins. Dent Mater 1985; 1: 11-14.

TABLES

Table 1.Mean (95% confidence intervals) of physicochemical properties tested.

Groups (*n*=10)	Water Sorption ($\mu g/mm^3$)	Solubility (mg/mm^3)	Degree of conversion %	Flexural Strength (MPa)
EO (Control)	180.2 (175.1 -185.3)	82.4 (79.3 - 85.5) [a]	68.5 (66.5 - 70,1)	18.7 (15.6 - 21.8)
EGCG 0.01% w/w	173.9 (165.3 - 182.5)	59.7 (53.3 -66.1)[b]	69.4 (67.5 - 71.3)	16.0 (13.9 - 18.1)
EGCG 0.1% w/w	180.0 (173.8 -186.2)	62.9 (57.5 - 68.3)[b]	70.6 (68.6 - 72.6)	18.5 (14.8 - 22.2)

*Distinct superscript letters indicate statistical difference in the same columns ($p<0.05$).

Capítulo 4

3.4 CAPÍTULO 4

Development and characterization of PLGA microparticles for controlled release of epigallocatechin-3-galate

Characterization of catechin-loaded polymeric microparticles

Authors: Jiovanne Rabelo NERI[a,b], Nadine Luisa Guimarães ALBUQUERQUE[b], Monica YAMAUTI[c], Francisco Fábio Oliveira de SOUSA[d*], Amanda Pontes Maia PIRES[e], Rinaldo dos Santos ARAÚJO[e] and Sérgio Lima SANTIAGO[b]

Affiliations:

[a]*Graduate School of Dentistry, University of Fortaleza, Fortaleza, Ceará, Brazil.*

[b]*Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.*

[c]*Department of Restorative Dentistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

[d]*School of Pharmacy, Department of Biological and Health Sciences, Federal University of Amapá, Macapá, Amapá, Brazil.*

[e]*Department of Chemistry and Environmental Technology, Federal Institute for Education, Science and Technology of Ceará, Fortaleza, Ceará, Brazil.*

*Corresponding author:

Dr. Francisco Fábio Oliveira de Sousa

Rod. Juscelino Kubitschek, km 02

68902-280

Macapa, AP - Brazil

E-mail: fabio@unifap.br

ABSTRACT

The aim of the study was to develop and characterize of poly (lactide-co-glycolide) acid (PLGA) micropartciles for controlled release of epigallocatechin-3-galate (EGCG), using two types of PLGA. A parallel stability study revealed that EGCG remained stable in solution over 50 days, independent of the concentration (5, 20 or 40 µg/mL) and storage condition (room temperature, incubator or freezer). PLGA 50:50/EGCG microparticles had spherical shape and smooth surfaces. Conversely, PLGA 75:25/EGCG microparticles did not presented spherical shape and had an irregular surface, some microparticles presented external voids. Microparticle size of PLGA 50:50/EGCG averaged 1.010 µm and PLGA 75:25/EGCG was 7.159 µm. The yield of production was 55.33 and 37.11% to PLGA50:50 /EGCG and PLGA75:25 /EGCG, respectively. PLGA 50:50/EGCG showed the encapsulation efficacy lower than PLGA 75:25/EGCG. At 24 h, PLGA 75:25/EGCG showed higher EGCG release (64.04%) than PLGA 50:50/EGCG (7.77%) ($p<0.001$). On the other hand, there was not statistical difference between the EGCG cumulative release values for PLGA 50:50/EGCG (95.13%) and PLGA 75:25 (100%) at 2952 h ($p=0.409$). Both microparticles showed a pulsatile drug release pattern, which would benefit the need of different peaks of action. It can be concluded that epigallocatechin-3-gallate loaded-PLGA microparticles could be a useful alternative for controlled release of this drug.

Keywords: catechin; polymers; drug delivery systems

1. INTRODUCTION

Green tea (Camellia sinensis) is one of the most popular beverages in the world, and its consumption is directly associated with beneficial effects on human health (Okello et al., 2015). Epigallocatechin-3-gallate is the principal component of green tea extract, accounting for about 48%-55% of the total catechins (Jun et al., 2010). This polyphenol has received considerable attention from the scientific community because it can interfere and control some pathological processes (Khurana et al., 2013).

Epigallocatechin-3-gallate is effective against cancer (Irimie et al.,2015), reduces the oxidative stress of cells and inflammation process (Liu et al., 2014), prevent cardiovascular diseases (Kim et al., 2010) and reduce the risk of neurodegenerative diseases (Mandel et al., 2004; Walker et al., 2015). In addition, It has been demonstrated that epigalocatechin-3-gallate prevents infectious diseases, such hepatitis B (Huang et al., 2014), caries (Xu et al, 2011) and periodontal disease (Asahi et al., 2014), by inhibition of some microorganisms activity (Reygaert, 2014). However, to produce therapeutic effects is necessary to guarantee the stability and bioavailability of epigallocatechin-3-gallate in the medium (Song et al., 2014).

The oral bioavailability of green tea catechins is low in humans, resulting in plasma concentrations 5 to 50 times less than concentrations shown to exert biological activities in *in loco* (Hong et al., 2001). When green tea is consumed, it is estimated that only 5% of the original dose of epigalocatechin-3-galate is absorbed and go to systemic circulation (Zhu et al., 2000). Two factors considered to be contributing to the limited oral bioavailability are the sensitivity of catechin to the digestive system and absorption barriers found in the human gastrointestinal tract (Song et al., 2014). In an attempt to improve the bioavailability of catechins in human organism has been researched protection of epigallocatechin-3-gallate molecules with polymers (Srivastava et al., 2013) and the application of this catechin *in situ* (Kato et al., 2010; Shin et al., 2014), both yield promising results.

The Poly (lactide-co-glycolide acid) blends are classic polymer used to develop drug delivery systems due to their superior biodegradability, biocompatibility and be approved by US FDA and European Medicine Agency, including for the invasive routes. (Li et al., 2008) The release of the drug occurs through contact of the microparticles with body fluids (Cappelano et al., 2014). The liquids penetrates into the microparticles, dissolves the drug, and causes polymer chain cleavage. Once dissolved, the drug starts to diffuse out into the surrounding bulk fluid (Gaignaux et al., 2012). The time degradation of poly (lactide-co-glycolide acid) and pattern of drug release depend on the molecular weight of polymer

(lactide:glicolide rates) and hydrophobicity (Makadia and Siegel, 2011). To the best of the current authors' knowledge, no studies exist that have examined the pattern release of catechin peformed with different types of poly (lactide-co-glycolide) acid.

Thus, the aim of the present study was to promote the development and characterization of PLGA micropartciles for controlled release of epigallocatechin-3-galate, using two types of PLGA. The null hypothesis tested was that there will be no statistical difference between the percentage of cumulative released of epigallocatechin-3-gallate after 24 and 2952 hours, regardless type of poly (lactide-co-glycolide acid).

2. MATERIALS AND METHODS

1. Materials

Poly (D-L lactide-co-glycolide) acid (PLGA): Resomer® RG502H (PLGA 50:50 - batch #STBD2887V) and Resomer® RG756S (PLGA 75:25 - batch #STBC6378V) were purchased from Sigma Aldrich, Germany. Epigallocatechin-3-gallate (EGCG - batch #SLBL1959V) and ethyl acetate (batch #DCBB6676) were purchased from Sigma Aldrich, United States of America. Dichloromethane (DCM - batch #65456) was obtained from Dinâmica, Brazil. All other chemicals were of analytical reagent grade and were used as received.

2. Analytical validation

2.1 Epigallocatechin-3-gallate quantification

Validation was performed to guarantee linearity, precision and accuracy in the measurements. Six concentrations, ranging from 2.5 to 30 µg/mL, were assayed using an UV-visible spectrophotometer (Evolution 60S, Thermo Scientific, Ohio, USA). The standard curve presented feasible within the assayed concentrations ($R^2 = 0.9997$).

2.2 EGCG stability in solution

A stability experiment was performed to evaluate the degradation kinetics in aqueous medium (Milli-Q water; pH 7.40) of this drug, after storage under different conditions. Concentrations of 5, 20 and 40 µg/mL of EGCG were measured after storage in room temperature (25 °C), incubator (37 °C) and freezer (4 °C). Solutions were measured at pre-set time intervals along 50 days to guarantee its practical usage.

3. Preparation of EGCG-load PLGA microparticules

Formulations were prepared in the ratio PLGA: EGCG of 16:1. Two different forms of PLGA was used: 50:50 and 75:25 as mentioned in the materials section. Due to differences in solubility among the drugs and polymer, an emulsification process was proposed. Briefly, PLGA 5.12% w/v was dissolved in DCM and EGCG 0.64% dissolved in ethyl acetate were mixed, under magnetic stirring for 10 min at 25°C using a high shear mixer (Ultraturrax IKA T10B; IKA/Works, Inc. NC, USA) at 19,000 rpm for 5 min.

Resulting PLGA/EGCG emulsion was immediately spray dried using a Büchi B-290 mini spray drier (Büchi Labortechnik AG, Flawil, Switzerland), according to Souza et al. (2010) All obtained EGCG-load PLGA microparticules were collected from the glass containers and stored at 4°C. A blank formulation (PLGA only) was obtained in the same manner and used as a reference.

4. Physical and chemical characterization of EGCG-loaded microparticles

4.1 Morphological and size determination

Microparticles were morphologically examined by scanning electronic microscopy (FE-SEM Ultra Plus; Carl Zeiss, Baden-Württemberg, Germany). Samples of dried microparticles were placed on double-sided carbon adhesive stickers and analyzed freshly in the apparatus.

Microparticles size and polydispersity index were determined by light scattering (Zetasizer Nano ZS, Malvern, Worcestershire, United Kingdom) in aqueous solution of Tween™ 20 (0.1%) (Sigma Aldrich, St. Louis, MO, USA). Measurements were made in triplicate.

4.2 Yield of production, Encapsulation efficacy and Drug loading

The yield of production of microparticles was determined according to the ratio between the obtained mass and theoretical mass of the polymer and drug used.

The amount of EGCG entrapped within the microparticles was determined using the solvent-separation method. For that, 10 mg of microparticles accurately weighted were added and dissolved in 0.4 mL of DCM, followed by the addition of 0.8 mL of Milli-Q water and stirred vigorously using a tube agitator (QL-901,Biomixer, São Paulo, SP, Brazil). The samples were centrifuged for 10 min at 10,000 rpm (NT810, Novatecnica, São Paulo, Brazil) to separate the two phases. The procedure was repeated until the complete extraction of the drug.

Encapsulation efficacy (EE) and Drug loading (DL) (n=3) were calculated using the following formulae:

$$EE\ (w/w\ \%) = \frac{M\ enc}{M\ 0} \qquad DL(w/w\%) = \frac{M\ enc}{M\ ms}$$

Where *M enc* is the real content of drug in the microparticles, *M 0* is the theoretical amount of drug in the formulation and *M ms* is the mass (mg) of microparticles used in the assay.

5. *In vitro* EGCG release studies

Five milligrams of EGCG-loaded microparticles (*n*=4) were placed in the glass vials in direct contact to the release medium (1.5 mL of Milli-Q water) and kept in an incubator (TE-392/1-MP; Tecnal Equipamentos para Laboratório, Piracicaba, SP, Brazil) at 37 °C. The release medium was collected at pre-setted times and measured immediately by a UV-Vis Spectrophotometer (DU-730; Beckman Coulter, Fullerton, CA, USA). Release medium was changed when release the concentration overcame the limit of standard curve values. Solutions were measured along over 4 months.

Statistical procedures were performed with the statistical software Sigmastat 3.5 (Systat Software Inc., San Jose, CA, USA) for Windows statistical program software. A Shapiro-Wilk test was applied to all groups to analyze the normal distribution of errors and the Barlett test for the homoscedasticity. To analyze EGCG cumulative release in 24 h and 2952 h was used Two-way ANOVA on Ranks (independent factors: PLGA blends and storage time). Post-hoc comparisons was analyzed by Holm-Sidak method. The level of significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Analytical validation

3.1.1 EGCG stability in solution

There is great controversy in the literature regarding the stability of catechins in an aqueous medium (Wang et al. 2008). It seems that storage temperature can affect the stability of tea catechins (Chen et al., 2001; Wang et al., 2008). Wang et al. (2000) showed that approximately 30% of total catechins was lost when green tea was kept at 40 °C for 6 months. Other studies (Wang et al., 2008; Cheng et al., 2001) observed that tea catechins degraded slowly at temperatures between 25 and 100ºC. Nevertheless, in the current study, EGCG levels remained stable over 50 days, independent of concentration (5, 20 or 40 µg/mL) and storage condition (room temperature, incubator or freezer) (Fig. 1).The degradation of epigallocatechin-gallate is commonly observed at high temperatures, above 70 °C (Wang et al., 2008).

3.2 Physical and chemical characterization of EGCG-loaded microparticles

3.3 Morphological and size determination

Particle size distribution and particle shape, together with some selected chemical properties, usually constitute the critical variables of a pharmaceutical manufacturing process (Shekunov et al, 2007). Spray dried particles are, normally, spherical, and their size can be described by their geometric diameter (Shekunov et al., 2007). In the current study, the micrographs showed that PLGA 50:50/EGCG microparticles had spherical shape and smooth surfaces (Fig. 2). However, PLGA 75:25/EGCG microparticles did not present spherical shape and had an irregular surface and, some microparticles presented external voids (Fig. 3).According to Vehring et al. (2008), external voids space can be created by process conditions and formulations that cause early separation of a soft surface layer, which folds to form a wrinkled morphology. The increased hidrofobicity of the polymer may have been crucial on the microparticles formation and caused a certain resistance on the polymer coating of the drug, which is majorly hydrophilic.

Particle size can be mensured because influence the dissolution rate, controlled release and bioavailability of active pharmaceutical ingredients (Burgess et al., 2004). Microparticle size of PLGA 50:50/EGCG averaged 0.780 µm and PLGA 75:25/EGCG was 7.159 µm (Table 1). As necessary as the average particle size is the analysis of size distribution of particles in a

product (Vehring, 2008). PLGA 75:25/EGCG presented high polydispersity index (0.980) (Table 1), indicating that microparticles were not uniform and homogeneous, as represented in Figure 3. On the orher hand, PLGA 50:50/EGCG showed homogeneous size of microparticles (Fig. 2), thus obtained a low PdI (0.219) (Table 1).

3.2.2. Yield of production, Encapsulation efficacy and Drug loading

The yield of production was 55.33 and 37.11% to PLGA50:50 /EGCG and PLGA75:25 /EGCG, respectively (Table 1). One limitation of spray dryer technique is the difficulty of recovering the atomized content (Zgoulli et al, 1999; Ambike et al., 2005). There may be a significant loss of the product during spray-drying process, due to adhesion of the microparticles to the inside wall of the spray-drier apparatus, and can also produce agglomeration of the microparticles (Takada et al., 1995). Low yield production observed in the PLGA75:25 /EGCG may be due to size and aggregation of particles (Fig. 3) that facilitated its sedimentation within the spray dryer chamber, making it difficult to collect further in the cyclone.

The encapsulation efficacy of the microparticles depends on different factors like concentration of the polymer, interaction between drug and polymer and solubility of polymer in solvent (Jyothi et al., 2010). PLGA 50:50/EGCG showed the encapsulation efficacy lower than PLGA 75:25/EGCG (Table 1). The increase in glycolic acid content in the poly (D-L lactide-co-glycolide) acid resulted in reduced solubility of the polymer in dichloromethane (Morishita and Park, 2010), and probably reduced the encapsulation efficacy of the PLGA 50:50/EGCG compared with PLGA 75:25/EGCG. The size may also have impacted the entrapment level observed within the different blends.

On the other hand, particles with maximal drug loading reduces the quantity of carrier required for the administration of sufficient amount of active compound (drug) to the target site as well as drug wastage during manufacturing (Govender et al., 1999). Both particles, PLGA 50:50/EGCG and PLGA 75:25/EGCG showed low levels of drug loading (Table 1).

3.4 *In vitro* EGCG release studies

There is a statistically significant interaction between microparticles types and release times ($p<0.001$; $F=17.611$). At 24 h, PLGA 75:25/EGCG showed higher EGCG release (64.04%) than PLGA 50:50/EGCG (7.77%) ($p<0.001$). On the other hand, there was not

statistical difference between the EGCG cumulative release values for PLGA 50:50/EGCG (95.13%) and PLGA 75:25 (100%) at 2952 h (p=0.409).

PLGA 50:50/EGCG presented three-step burst release effect, at 96 h (21.15%), 360 h (61.56%) and 2952 h (95.13%), while the PLGA 75:25/EGCG showed a different pattern release with two-step burst release at 48 h (70.91%) and 504 h (100%) (Fig. 4A). Although, both microparticles showed a pulsatile drug release, PLGA 50:50/EGCG presented more controlled release pattern over the experimental period as evidenced by drug amount release at each interval. Additionally, PLGA 50:50/EGCG showed a delayed release after 2256 h (Fig. 4B).

Recently, pulsatile drug delivery systems are gaining a lot of interest and attention (Mangubat et al., 2015; Sha et al., 2015). A major objective of controlled delivery systems in the treatment of several diseases is to deliver the drug in higher concentrations during the time of greatest need according to the onset of the disease or syndrome (Pragna et al., 2013). Some researches have used the epigallocatechin-3-gallate associated with polymers, such drug delivery system, to treatment of atherosclerosis in rabbits (Hong et al., 2014), to prevent postsurgical adhesion (Shin et al., 2014), caries (Mankovskaia et al., 2013) and pancreatic cancer (Sun et al., 2014). In this context, PLGA 50:50/EGCG and PLGA 75:25/EGCG microparticles could be an alternative for use in Medicine and Dentistry.

4. CONCLUSION

Based on all experimental results, it can be concluded that epigallocatechin-3-gallate-loaded poly (D-L lactide-co-glycolide) acid microparticles, especially the ones based in PLGA 50:50, could be a useful tool for controlled drug release.

ACKNOWLEDGEMENTS

This work was funded thanks to grants CNPq 140058/2013-3 and CNPq 140073/2014-0. FUNCAP CI1-0800-000540100/13

REFERENCES

Abdelwahed, W., Degobert, G., Stainmesse, S., Fessi, H. 2006. Freeze-drying of nanoparticles: formulation, process and storage considerations. Adv. Drug. Deliv. Rev. 58, 1688-1713.

Adler, M.; Lee, G. 1999. Stability and surface activity of lactate dehydrogenase in spray - dried trehalose. J. Pharm. Sci. 88, 199 -208.

Ambike, A. A., Mahadik, K. R., Paradkar, A. 2005. Spray-dried amorphous solid dispersions of simvastatin, a low tg drug: in vitro and in vivo evaluations. Pharm. Res. 22, 990-998.

Asahi, Y., Noiri, Y., Miura, J., Maezono, H., Yamaguchi, M., Yamamoto, R., Azakami, H., Hayashi, M., Ebisu, S. 2014. Effects of the tea catechin epigallocatechin gallate on Porphyromonas gingivalis biofilms. J. Appl. Microbiol. 116, 1164-1171.

Burgess, D. J., Duffy, E., Etzler, F., Hickey, A. J. 2004. Particle size analysis: AAPS workshop report, cosponsored by the Food and Drug Administration and the United States Pharmacopeia. AAPS J. 6, 20.

Cappellano, G., Woldetsadik, A. D., Orilieri, E., Shivakumar, Y., Rizzi, M., Carniato, F., Gigliotti, C. L., Boggio, E., Clemente, N., Comi, C., Dianzani, C., Boldorini, R., Chiocchetti, A., Renò, F., Dianzani U. 2014. Subcutaneous inverse vaccination with PLGA particles loaded with a MOG peptide and IL-10 decreases the severity of experimental autoimmune encephalomyelitis. Vaccine. 32, 5681-5689.

Chen, Z., Zhu, Q. Y., Tsang, D., Huang, Y. 2001. Degradation of green tea catechins in tea drinks. J. Agric. Food Chem. 49, 477-482.

De, S., Robinson, D. H. 2004. Particle size and temperature effect on the physical stability of PLGA nanospheres and microspheres containing Bodipy. AAPS Pharm. Sci. Tech. 5, 53.

Gaignaux, A., Réeff, J., Siepmann, F., Siepmann, J., De Vriese, C., Goole, J., Amighi, K. 2012. Development and evaluation of sustained-release clonidine-loaded PLGA microparticles. Int. J. Pharm. 437, 20-28.

Govender, T., Stolnik, S., Garnett, M. C., Illum, L., Davis, S. S. 1999. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J. Control. Release. 5, 171-185.

Hong, J., Smith, T. J., Ho, C. T., August, D., Yang, C. S. 2001. Effects of purified green and black tea polyphenols on cyclooxygenase- and lipoxygenase-dependent metabolism of

arachidonic acid in human colon mucosa and colon tumor tissues. Biochem. Pharmacol. 62, 1175–1183.

Hong, Z., Xu, Y., Yin, J. F., Jin, J., Jiang, Y., Du, Q. 2014. Improving the effectiveness of (-)-epigallocatechin gallate (EGCG) against rabbit atherosclerosis by EGCG-loaded nanoparticles prepared from chitosan and polyaspartic acid. J. Agric. Food Chem. 62, 12603-12609.

Huang, H.C., Tao, M. H., Hung, T. M., Chen, J. C., Lin, Z. J., Huang, C. 2014. (-)-Epigallocatechin-3-gallate inhibits entry of hepatitis B virus into hepatocytes. Antiviral. Res. 111, 100-111.

Irimie, A. I., Braicu, C., Zanoaga, O., Pileczki, V., Gherman, C., Berindan-Neagoe, I., Campian, R. S. 2015. Epigallocatechin-3-gallate suppresses cell proliferation and promotes apoptosis and autophagy in oral cancer SSC-4 cells. Onco. Targets Ther. 20, 461-470.

Jun, X.; Shuo, Z.; Bingbing, L.; Rui, Z.; Ye, L.; Deji, S.; Guofeng, Z. 2010. Separation of major catechins from green tea by ultrahigh pressure extraction. Int. J. Pharm. 386, 229-231.

Jyothi, N. V., Prasanna, P. M., Sakarkar, S. N., Prabha, K. S., Ramaiah, P. S., Srawan, G. Y. 2010. Microencapsulation techniques, factors influencing encapsulation efficiency. J. Microencapsul. 27, 187-197.

Kato, M. T., Leite, A. L., Hannas, A. R., Buzalaf, M. A. 2010. Gels containing MMP inhibitors prevent dental erosion in situ. J. Dent. Res. 89, 468-472.

Khurana, S., Venkataraman, K., Hollingsworth, A., Piche, M., Tai, T. C. 2013. Polyphenols: benefits to the cardiovascular system in health and in aging. Nutrients. 5, 3779-3827.

Kim, C. J., Kim, J. M., Lee, S. R., Jang, Y. H., Kim, J. H., Chun, K. J. 2010. Polyphenol (-)-epigallocatechin gallate targeting myocardial reperfusion limits infarct size and improves cardiac function. Korean J. Anesthesiol. 58, 169-175.

Li, M., Rouaud, O., Poncelet, D. 2008. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. Intern. J. Pharm. 363, 26-39.

Liu, P. L., Liu, J. T., Kuo, H. F., Chong, I. W., Hsieh, C. C. 2014. Epigallocatechin gallate attenuates proliferation and oxidative stress in human vascular smooth muscle cells induced by interleukin-1β via heme oxygenase-1. Mediators Inflamm. 523684.

Makadia, H. K., Siegel, S. J. 2011. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers (Basel). 3, 1377-1397.

Mangubat, E. Z., Kellogg, R. G., Harris, T. J. Jr., Rossi, M. A. 2015. On-demand pulsatile intracerebral delivery of carisbamate with closed-loop direct neurostimulation therapy in an electrically induced self-sustained focal-onset epilepsy rat model. J. Neurosurg. 27,1-10.

Mandel, S., Weinreb, O., Amit, T., Youdim, M. B. 2004. Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. J. Neurochem. 88, 1555-1569.

Mankovskaia, A., Lévesque, C. M., Prakki, A. 2013. Catechin-incorporated dental copolymers inhibit growth of Streptococcus mutans. J. Appl. Oral Sci. 21, 203-207.

Morishita, M. - Park, K. Biodrug Delivery Systems: Fundamentals, Applications and Clinical Development. 1ª ed, 2009, Anja Vetter and Andreas Bernkop-Schnürch

Okello, E. J., Abadi, A. M., Abadi, S. A. 2015. Effects of green and black tea consumption on brain wave activities in healthy volunteers as measured by a simplified Electroencephalogram (EEG): A feasibility study. Nutr. Neurosci. 25. http://dx.doi.org/10.1179/1476830515Y.0000000008

Pragna, G., Shravani, B., Raghavendra Rao, N. G. 2013. Pulsatile drug delivery system: an overview. Inter. J. Pharm. Devel. Tech.3, 97-105.

Reygaert, W. C. 2014. The antimicrobial possibilities of green tea. Front. Microbiol. 20, 434.

Shah, S., Patel, R., Soniwala, M., Chavda, J. 2015. Development and optimization of press coated tablets of release engineered valsartan for pulsatile delivery. Drug. Dev. Ind. Pharm. 27, 1-12.

Shekunov, B. Y., Chattopadhyay, P., Tong, H. H., Chow, A. H. 2007. Particle size analysis in pharmaceutics: principles, methods and applications. Pharm. Res. 24, 203-227.

Shin, Y. C., Yang, W. J., Lee, J. H., Oh, J. W., Kim, T. W., Park, J. C., Hyon, S. H., Han, D. W. 2014. PLGA nanofiber membranes loaded with epigallocatechin-3-O-gallate are beneficial to prevention of postsurgical adhesions. Int. J. Nanomedicine. 9, 4067-4078.

Song, Q., Li, D., Zhou, Y., Yang, J., Yang, W., Zhou, G., Wen, J. 2014. Enhanced uptake and transport of (+)-catechin and (-)-epigallocatechin gallate in niosomal formulation by human intestinal Caco-2 cells. Int. J. Nanomedicine. 9, 2157-2165.

Sousa, F. F., Luzardo-Alvarez, A., Pérez-Estévéz, A., Seoane-Prado, R., Blanco-Méndez, J. 2010. Development of a novel AMX-loaded PLGA/zein microsphere for root canal disinfection. Biomed. Mater. 5, 055008.

Sun, L., Zhang, C., Li, P. 2014. Copolymeric micelles for delivery of EGCG and cyclopamine to pancreatic cancer cells. Nutr. Cancer. 66, 896-903.

Takada, S., Uda, Y., Toguchi, H., Ogawa, Y. 1995. Application of a spray drying technique in the production of TRH-containing injectable sustained-release microparticles of biodegradable polymers. PDA J. Pharm. Sci. Technol. 49, 180-184.

Vehring, R. 2007. Pharmaceutical particle engineering via spray drying. Pharm. Res. 25, 999-1022.

Walker, J. M., Klakotskaia, D., Ajit, D., Weisman, G. A., Wood, W. G., Sun, G. Y., Serfozo, P., Simonyi, A., Schachtman, T. R. 2015. Beneficial effects of dietary EGCG and voluntary exercise on behavior in an Alzheimer's disease mouse model. J. Alzheimers Dis. 44, 561-572.

Wang, R., Zhou, W., Jiang, X. 2008. Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range. J. Agric. Food Chem. 56, 2694-2701.

Xu, X., Zhou, X. D., Wu, C. D. 2011. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of Streptococcus mutans. Antimicrob. Agents Chemother. 55, 1229-1236.

Zhu, M.; Chen, Y.; Li, R.C. 2000. Oral absorption and bioavailability of tea catechins. Planta Med. 66, 444–447.

Zgoulli, S., Grek, V., Barre, G., Goffinet, G., Thonart, P., Zinner, S. 1999. Microencapsulation of erythromycin and clarithromycin using a spray-drying technique. J. Microencapsul. 16, 565-571.

TABLE

Table 1. Main characteristics of EGCG loaded-PLGA microparticles.

Microparticles	Yield of production (%)	Particle size (μm) (PdI)	Encapsulation efficacy (%)	Drug loading (%)
PLGA 50:50	44.91	1.010 (0.219)	-	-
PLGA50:50 /EGCG	55.38	0.780 (0.687)	42.35	2.49
PLGA 75:25	48.31	5.832 (0.538)	-	-
PLGA 75:25 / EGCG	37.11	7.159 (0.980)	100	6.03

FIGURES

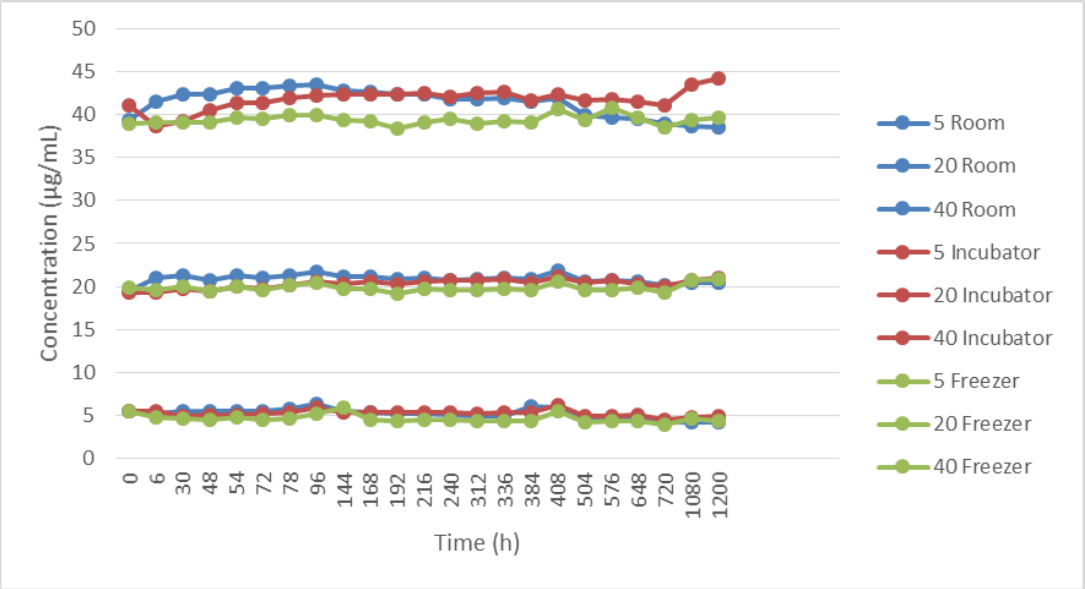


Fig. 1. Stability in solution of EGCG; where 5, 20 and 40 refere to the concentration in µg/mL, after storage under different conditions: room (25°C), incubator (37°C) and freezer (4°C) up to 50 days.

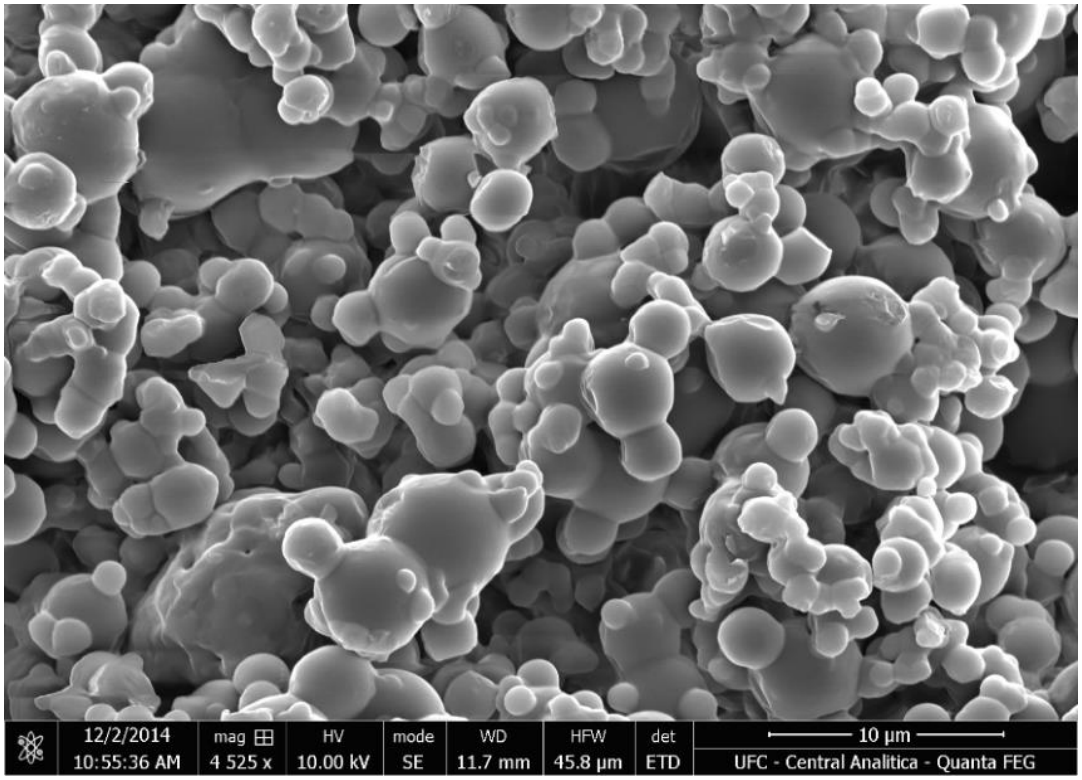


Fig 2. SEM image of PLGA 50:50/EGCG microparticles

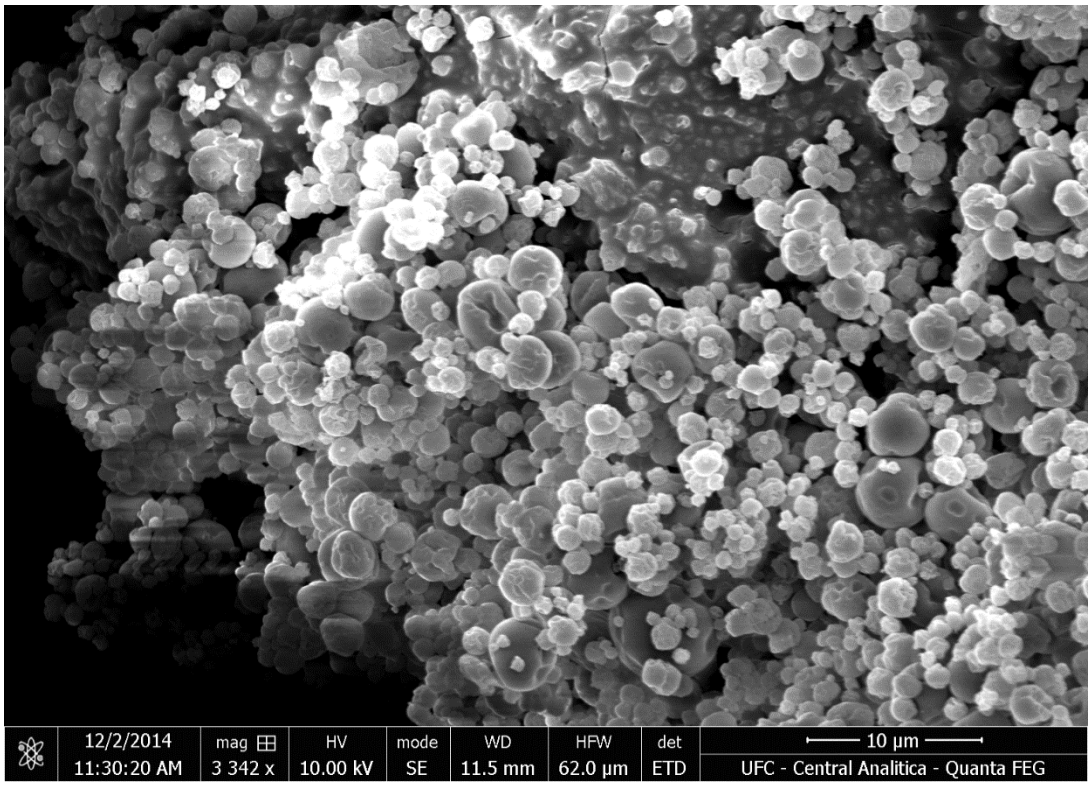
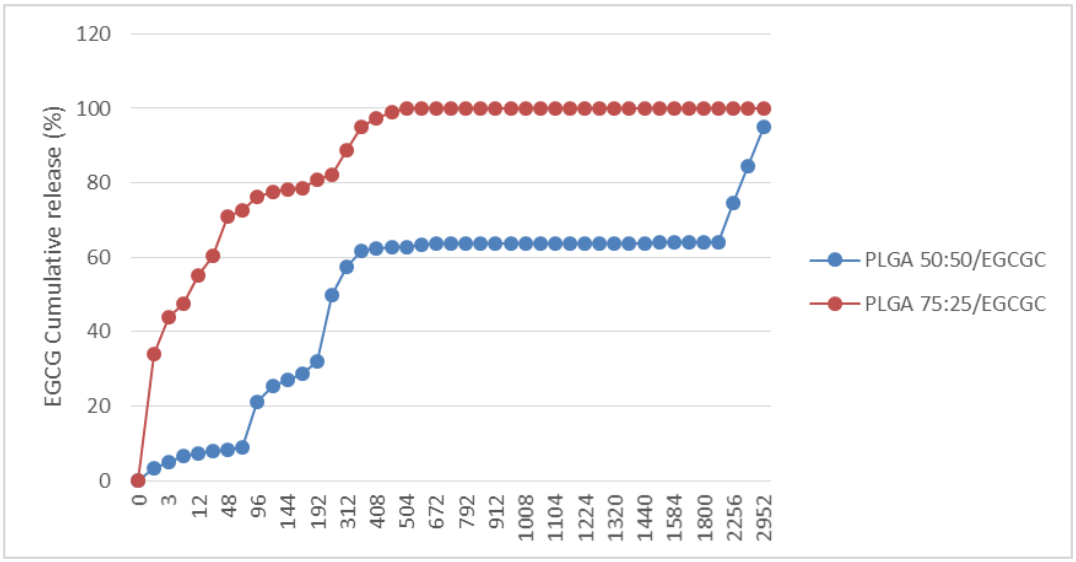


Fig. 3. SEM image of PLGA 75:25/EGCG microparticles

(A)



(B)

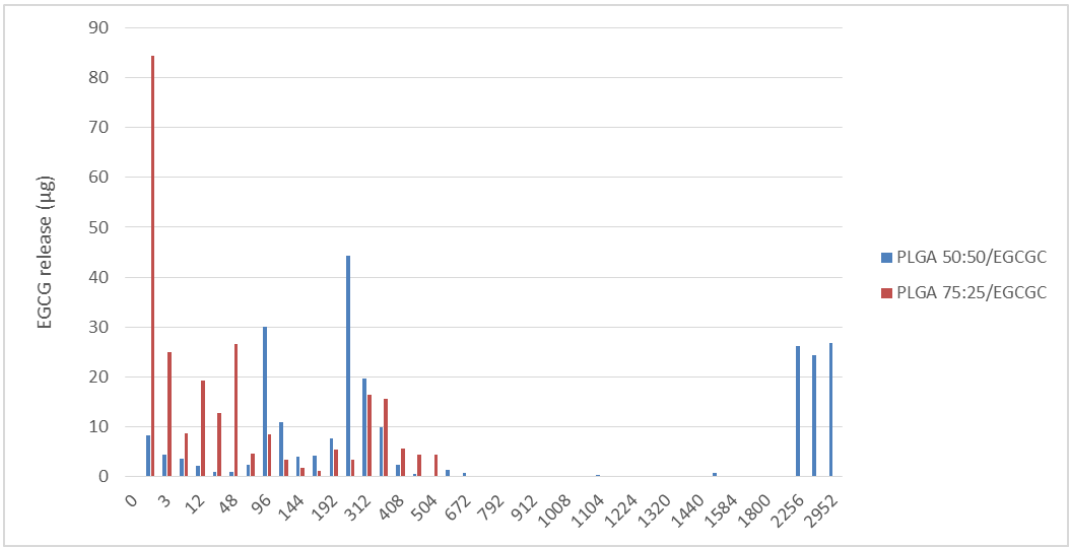


Fig. 4. *In vitro* EGCG release studies: EGCG profiles of PLGA microparticles (A); EGCG release over time from PLGA microparticles (B)

Capítulo 5

3.5 CAPÍTULO 5

Physicochemical properties and pattern of drug release of etch-and-rinse adhesive system incorporated with catechin-loaded polymeric microparticles

Running title: Dental adhesive doped with EGCG-loaded microparticles

Authors: Jiovanne Rabelo NERI[a,b], Nadine Luisa Guimarães ALBUQUERQUE[b], Marcelo Sidou LEMOS[b], Monica YAMAUTI[c], Francisco Fábio Oliveira de SOUSA[d], and Sérgio Lima SANTIAGO[b]

Affiliations:

[a]*Graduate School of Dentistry, University of Fortaleza, Fortaleza, Ceará, Brazil.*

[b]*Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.*

[c]*Department of Restorative Dentistry, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.*

[d]*Department of Pharmaceutical Sciences, Federal University of Amapá, Macapá, Amapá, Brazil.*

*Corresponding author:

Dr. Sérgio Lima Santiago

Rua Monsenhor Furtado S/Nº

60430-355

Fortaleza, CE - Brazil

E-mail: sergiosantiago@ufc.br

Tel: +558588242704

Fax: +558533668232

ABSTRACT

Objective: This study aimed to evaluate the epigallocatechin-3-gallate-load poly (lactide-co-glycolide) acid (PLGA) microparticules incorporation on the physicochemical properties of a two-step etch-and-rinse adhesive system, and the release rate of epigallocatechin-3-gallate (EGCG).

Methods: EGCG was added to the Single Bond 2, directly in 0.01% w/w (EGCG 0.01%) and 0.1% w/w (EGCG 0.1%) concentrations or microencapsulated with PLGA 50:50 or PLGA 75:25 microparticles in 0.5, 1, and 2% w/w. In phase 1, was evaluated EGCG cumulative release (CR) using an UV-Vis Spectrophotometer. In phase 2, degree of conversion (DC) was analyzed by FTIR whereas flexural strength (FS) and elastic modulus (E) were tested in three-point bending with bar specimens (n=10). For water sorption (WS) and solubility (SL) surveys, resin discs were assayed following ISO recommendations (n=10). Specimens sticks-shaped were used to evaluate the resin-dentin bond strength (μTBS). One-way ANOVA was used to DC, FS, E, WS, SL, μTBS and for analyze EGCG release was used Two-way ANOVA on Ranks. Comparisons post hoc was analyzed by Holm-Sidak method. The level of significance was set at $p<0.05$.

Results: 1% PLGA50:50/EGCG achieved d the highest release in quantitative terms reaching the highest released mass (77.30 μg).There was no significant difference in the DC, E, WS, SL and μTBS values among all groups ($p>0.05$). FS values were significantly increased in the 1% PLGA50:50/EGCG ($p<0.05$).

Conclusions: Single Bond 2 doped with EGCG-load PLGA 50:50 microparticules at 1% could be an alternative to promote controlled release without causing detrimental effects of physicochemical properties.

Clinical Relevance: Adhesives systems doped with epigalocatechin-3-gallate, directly or in PLGA microparticles, do not cause detrimental effect of restorative procedures.

Keywords: Catechin; Dentin; Physical properties; Dental adhesive; Drug delivery systems

INTRODUCTION

Biomodification of demineralized collagen matrices with cross-linking agents has attracted the attention of researchers in recent years.[1-4] The use of bioactive agents during the bonding procedures improves the biomechanical properties of dentin by increasing the tensile strength and modulus of elasticity of the collagen fibrils.[1,5] Moreover, most strategies of dentin biomodification reduces the proteolytic activity of enzymes, such as cysteine cathepsins (CTs)[4,6] and matrix metalloproteinases (MMPs),[4,7,8] that degrade the collagen fibrils of hybrid layer. Therefore, dentin biomodification agents may have an important role on the preservation of resin-dentin interfaces.[2]

A large variety of bioactive agents can be found in the plants.[1,4,8] Of particular interest to the adhesive dentistry is the epigallocatechin-3-gallate, the principal flavonoid of green tea (*Camelia sinesis*).[3,9-11] This catechin prevents episodes of dental demineralization and inhibits the progress of caries in dentin.[12] In addition, epigallocatechin-3-gallate is an effective inhibitor of activity of MMP-2 and MMP-9[4,8] and CT-B.[4,6] It has been shown that epigallocatechin-3-gallate can increase the collagen cross-linking and prevent the free access of collagenase to the active sites on the collagen chains.[13]

Recent studies demonstrated that the use of epigallocatechin-3-gallate at 0.01%[9] and 0.1%[3] were effective in preserving resin-dentin bond strength up to 6 months. However, there is concern about the ability of adhesive systems to release the catechin for more long periods.[10] The release of epigallocatechin-3-gallate, in water, when incorporated directly into polymeric materials was significantly reduced after 24 hours.[10] Thus, the performance of epigallocatechin-3-gallate as an inhibitor of the enzyme activity could be insufficient to inhibit the enzymatic activity and maintain the bond strength after a few years.

The controlled release may be obtained by entrapping the epigallocatechin-3-gallate in biodegradable microparticles of poly (lactide-co-glycolide) acid.[14] Poly (lactide-co-glycolide) acid is the most used polymer to develop drug delivery systems due to be biodegradable, biocompatible and approved by US FDA.[15] The pattern of drug release may be affected by the composition of poly (lactide-co-glycolide) acid, since the poly lactic acid is more hydrophobic than poly glycolic acid.[16] The release of the drug occurs through contact of the microparticles with aqueous body.[17] Water molecules penetrates into the microparticles, dissolves the drug, and causes polymer chain cleavage. Once dissolved, the drug starts to diffuse out into the surrounding bulk fluid.[18] Although the use of drug delivery systems seem promising, little is known about the consequences of their incorporation in the properties of adhesive systems.

Therefore, this study was designed to evaluate the effect of the epigallocatechin-3-gallate-load poly (lactide-co-glycolide) acid (PLGA) microparticules incorporation on the physicochemical properties of a two-step etch-and-rinse adhesive system. To achieve this objective, the study was divided into two phases. The aim of the phase 1 was to select the more effective EGCG pattern release in different types of poly (lactide-co-glycolide) acid microparticles incorporated in the two-step etch-and-rinse adhesive systems. On the other hand, the aim of phase 2 was to evaluate the physicochemical properties of a two-step etch-and-rinse adhesive system incorporated with epigallocatechin-3-gallate, directly or in poly (lactide-co-glycolide) acid microparticules, selected in the first phase. The first null hypothesis was that there is no significant difference between the groups regarding the EGCG release of adhesives systems, after 24 and 4320 hours. The second null hypothesis to be tested was that different incorporation modes of EGCG have no effect on the physicochemical properties.

MATERIAL and METHODS

1. Materials

Poly (D-L lactide-co-glycolide) acid (PLGA): Resomer® RG502H (PLGA 50:50 - batch #STBD2887V) and Resomer® RG756S (PLGA 75:25 - batch #STBC6378V) were purchased from Sigma Aldrich, Germany. Epigallocatechin-3-gallate (EGCG - batch #SLBL1959V) and ethyl acetate (batch #DCBB6676) were purchased from Sigma Aldrich, United States of America. Dichloromethane (DCM - batch #65456) was obtained from Dinâmica, Brazil. All other chemicals were of analytical reagent grade and were used as received.

2. Preparation of EGCG-load PLGA microparticules

Formulations were prepared in the ratio PLGA: EGCG of 16:1. Two different forms of PLGA were used: 50:50 and 75:25 mentioned in materials. Due to differences in solubility among the drugs and polymer, an emulsification process was proposed. Briefly, PLGA 5.12% w/v was dissolved in DCM and EGCG 0.64% dissolved in ethyl acetate were mixed, under magnetic stirring for 10 min at 25°C using a high shear mixer (Ultraturrax IKA T10B; IKA/Works, Inc. NC, USA) at 19,000 rpm for 5 min.

Resulting PLGA/EGCG solution was immediately spray dried using a Büchi B-290 mini spray drier (Büchi Labortechnik AG, Flawil, Switzerland), according to Souza *et al.*[19]. All obtained EGCG-load PLGA microparticules was collected in glass containers and stored in desiccators at 4 °C.

A blank formulation (PLGA only) was obtained and used as a reference.

3. Doping the adhesive with EGCG

A commercial two steps etch-and-rinse adhesive system, Adper Single Bond 2 (SB, 3M ESPE, St. Paul, MN, USA), was used in this study (Table 1). EGCG was added to the adhesive system in different incorporation modes, directly or microencapsuleted, according to Figure 1. In

order to obtain a homogenous mixture, the EGCG-doped adhesives were shaken using a tube agitator (QL-901,Biomixer, São Paulo, SP, Brazil) in darkness for 1 min. The homogeneity of the dilution was carefully checked and it was used only if no crystals were noted.

4. Release assay of adhesives containing EGCG (Phase 1)

In view of the difference in hydrophobicity and biodegradation rates of the PLGAs (PLGA 50:50 and PLGA 75:25) used in this study, there was a release assay in order to observe their performance into the adhesive system (Adper Single Bond 2).

A series of reference solutions ranging from 2.5 to 40 µg/mL of EGCG in distilled water were prepared to obtain a linear relationship between absorbance peak height and drug concentration. A UV-Vis Spectrophotometer (DU-730; Beckman Coulter, Fullerton, CA, USA) was used to evaluate and confirm the absorbance peak of EGCG at 275 nm. The standard curve performance was within acceptable range for bioanalytical method acceptance ($R^2 = 0.99982$).

Nine disc-shaped specimens (6.0 mm diameter and 1.0 mm thickness) of each adhesive system were prepared by using a silicon matrix. A Mylar strip and a glass slide were placed on the discs after dispensing the adhesive systems using micropipettes. The light-activation was undertaken for 40 s using a light source (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA) with 600 mW/cm² irradiance at both sides of each specimen and they were ground and slowly polished up to a thickness of 0.5 mm using 600-grit SiC polishing papers.

Discs were divided in three glass containing 1 ml of distilled water at 37 °C and stored until 180 days (4320 hours). The absorbance peaks heights of storage solutions were analyzed with a UV-Vis Spectrophotometer and converted to drug release rates based on the established linear calibration. The absorbance of blank microparticles was used to eliminate the polymer influence.

The adhesive system incorporated with PLGA microparticles loaded with EGCG that showed a pulsatile profile and highest mass released was selected for phase 2 of this study.

5. Analysis of the physicochemical properties of adhesive systems (Phase 2)

Four adhesive systems (SB - Control, EGCG 0.01%, EGCG 0.1% and 1% PLGA/EGCG) were evaluated *in vitro* using water sorption, water solubility, degree of conversion, flexural strength, elastic modulus, and microtensile bond strength experiments.

5.1.Degree of conversion

The degree of conversion (DC) of the adhesive resins was assessed by Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer Spectrum 100, Perkin Elmer, Shelton, CT, USA). Each adhesive system was dispensed into a small agate mortar and thoroughly mixed with potassium bromide (KBr) using a pestle, at a ratio of 4:100 w/w. The pellets of KBr/adhesive solution were prepared with a hand press (Hand Press Kit 161-1100, PIKE Technologies, Madison, WI, USA). FTIR spectrum of the uncured adhesive was obtained from each sample using 32 scans in a range of 4000-400 cm^{-1},at 4 cm^{-1} resolution in transmission mode.

The adhesive resins were light-activated for 20 s using the light source (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA). Additional FTIR spectra were obtained immediately after light curing. The analyses were performed at 25 °C with 70% relative humidity. Ten specimens per group (n=10) were tested. The rate of unreacted carbon-carbon double bonds (C=C) was determined from the ratio of absorbance intensities of aliphatic C=C (peak at 1636 cm^{-1}) against an internal standard (aromatic carbon-carbon bond peak at 1608 cm^{-1}) before and after curing. Degree of conversion was determined by subtracting the C=C from 100%.

5.2.Flexural Strength and Elastic Modulus

A three-point bending test was used to assess the flexural strength (FS) and elastic modulus (E) of bar-shaped specimens following ISO 4049:2000 except for specimen dimensions that were adapted for the microflexural test as previously undertaken by Gaglione *et al.*[20]

Each adhesive system was placed into a silicon matrix to prepare the bar-shaped specimens (7.0 mm length x 2.0 mm width x 1.0 mm height). The adhesive systems were following air-dried for 40 s for solvent evaporation and light-activated for 40 s with the light source (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA).

After light activation, the specimens were removed from the matrix, and the bottom surface underwent additional light-activation for 40 s. Specimens were stored for 24 h in distilled water at 37 °C and subjected to a three-point bending test using a universal testing machine (Instron 3345, Instron Corp., Canton, MA, USA) at a crosshead speed of 1.0 mm/min. Prior the test, the dimensions of each specimen were captured using a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm and recorded with Bluehill 2 software (Instron Corp., Canton, MA, USA), which calculated FS (MPa) and E (GPa) values, according dimensions and tension.

5.3.Water sorption/solubility

Water sorption and solubility were determined following ISO 4049:2000 except for specimen dimensions as previously undertaken by Collares *et al*.[21]

Ten disc specimens (6.0 mm diameter and 1.0 mm thickness) were prepared as previously described in EGCG release test. The discs were stored in a silica-containing desiccator at 37°C and were repeatedly weighed after a 24 h intervals on an analytical balance (AUX-220, Shimadzu, Tokyo, Japan) with an accuracy of 0.0001 g up to a constant mass (m1) was obtained (i.e.,variation less than 0.1 mg in three weight measures). The volume of each specimen were measured with a 0.001 mm precision digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) by analyzing the diameter and thickness, and the volume (V) was expressed in mm^3. Thereafter, the specimens were stored in sealed glass vials with 1.5 ml of distilled water at 37°C for 7 days. Afterwards, the specimens were weighed after gently wiped on absorbent papers to obtain a constant mass (m2) and then they were returned to the desiccator. The specimens were finally weighed as aforementioned up to stabilization of a constant mass (m3). Water sorption (WS) and solubility (SL) ($\mu g/mm^3$) were calculated using the following formulae:

$$WS = \frac{m2 - m3}{V} \qquad SL = \frac{m1 - m3}{V}$$

6. Microtensile Bond Strength Test (μTBS)

Thirty six unerupted, caries-free third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the local Research and Ethics Committee (# 459.659). The selected teeth were stored in 0.01% thymol solution and used within one month after extraction. Occlusal enamel was removed using a diamond saw in a Labcut 1010 (Extec, Enfield, CT, USA) under water-cooling to expose a flat coronal dentin surface. The dentin surface was then prepared with a wet #600-grit SiC paper for 60 s to standardize the bonding surface.

The teeth were randomly allocated by the Excel software (Excel 2013, Microsoft Corporatin, One Microsoft Way, Redmond, WA, USA) into 4 groups (n=9) according to the adhesive system used. The exposed dentin surfaces of all teeth were etched with 35% phosphoric acid gel (Scotchbond Phosphoric Acid Etchant; 3M ESPE, St. Paul, MN, USA - batch #1219600378) for 15 s, rinsed for 30 s with distilled water, and dried with oil-/water-free air for 30 s. The teeth were re-hydrated with 20 µL of distilled water and excess solution was removed with absorbent paper, leaving the dentin surface visibly moist. The etch-and-rinse adhesive system Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) was applied according to manufacturer's instructions (Table 1). After light curing the adhesive (Ellipar Freelight 2, 3M ESPE, St. Paul, MN, USA), five 1 mm thick increments of composite resin were built up (Filtek Z250XT; 3M ESPE, St. Paul, MN, USA - batch #37277). Each increment was light-cured (Ellipar Freelight 2) for 20 s, with a power density of 600 mW/cm^2. The bonded teeth were stored in distilled water at 37°C for 24 h.

After storage, the bonded teeth were longitudinally sectioned in both “x” and “y” directions across the bonded interface using a diamond saw in a Labcut 1010 (Extec, Enfield, CT, USA) under water cooling to obtain bonded sticks with cross-sectional area of approximately 1.0 mm^2. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm and recorded for subsequent calculation of bond strength values.

Sticks from each tooth were randomly allocated by the Excel software (Excel 2013, Microsoft Corporatin, One Microsoft Way, Redmond, WA, USA) and assigned to three storage times: 24 hours, 6 months and 12 months. For microtensile test, 24 hours bonded sticks were attached with cyanoacrylate glue (Super Bonder Flex Gel, Loctite, São Paulo, SP, Brazil) to a modified Geraldeli testing apparatus (Odeme Biotechnology, Joaçaba, SC, Brazil) and subjected to a tensile force at 0.5 mm/min in a universal testing machine (Emic, São José dos Pinhais, PR, Brazil). The load at fracture was used to calculate bond strength (MPa). Specimens of 6 and 12 months remain stored for later testing.

The failure modes were evaluated at 80 X magnification (StereoZoom® Leica S8 APO, Leica Microsystems, Wetzlar, Germany) and classified as cohesive when they were located exclusively within dentin (CD) or resin composite (CR); adhesive (A) when failure occurred at the dentin/adhesive interface; or mixed (M) when two modes of failure occurred simultaneously.

7. Statistical analysis

Statistical procedures were performed with the Sigmastat 3.5 (Systat Software Inc., San Jose, CA, USA) for Windows statistical program software. A Shapiro-Wilk test was applied to all groups to analyze the normal distribution of errors and the Barlett test for the homoscedasticity. For analyze cumulative EGCG release in 24 h and 4320 h was used Two-way ANOVA on Ranks (independent factors: storage time and adhesive system).One-way ANOVA was used to compare, GC, FS, E, WS, SL and μTBS within each adhesive system. Comparisons *post hoc* was analyzed by Holm-Sidak method. The level of significance was set at $p<0.05$. Teeth were used as a statistical unit and the number of prematurely debonded specimens was recorded, although this was not included in the analysis.

RESULTS

EGCG release profiles are shown in Figures 2 and 3. In phase 1, mean of EGCG cumulative release (%) were significantly influenced by adhesive system ($p<0.001$; $F=9.399$) and storage time ($p<0.001$; $F=140.924$). The interactions between variables were significant ($p<0.001$; $F=5.909$). At 24 h, there was no significant difference between EGCG release values among groups ($p>0.05$). After 4320 h, there was no significant difference among 0.5% PLGA 50:50/EGCG, 1% PLGA50:50/EGCG and EGCG 0.01% ($p>0.05$), and these adhesive systems reached total release (100%) during the assayed period. On the other hand, among the PLGA75:25 groups, the highest release obtained did not reach more than 68% of the overall drug content (0.5% PLGA75:25/EGCG).

All groups incorporated with PLGA microparticles showed a pulsatile release profile, characterized by moments of cessation and subsequent rapid increase of release. The 1% PLGA50:50/EGCG group presented four-step burst release effect, at 288 h (42.62%), 528 h (63.00%) 768 h (79.67%) and 1200 h (100%) (Figure 2). Additionally, 1% PLGA50:50/EGCG achieved the highest release in quantitative terms reaching the highest released mass (77.30 µg), among all studied groups and completing the full release at the end of 50 days (Figure 3). In contrast, the 0.5% PLGA 50:50/EGCG group showed the lowest release (39.84 µg) (Figure 3).

Table 2 showed DC, FS, E, WS and SL means values and standard deviation for adhesive systems. In the phase 2, there was no significant difference in the DC, E, WS and SL values among all groups ($p>0.05$). However, FS results were influenced by treatment ($p=0.009$; $F=4.432$). The incorporation of 1% PLGA 50:50/EGCG significantly increased the flexural strength ($p<0.05$). While the FS mean values for other groups were not statistically different ($p>0.05$).

Mean µTBS values are displayed in Table 3. After 24 h of storage, there was not statistically significant difference between the mean bond strength values of all groups ($p>0.05$). At 24 h, most of failures were mixed in all of the tested groups and, and cohesive failures (resin and dentin) were more prevalent than adhesive failures for specimens of all groups, except to SB group (control) (Table 4).

DISCUSSION

The idea of using adhesives systems as vehicles for the delivery of therapeutic agents is attractive[22]. Many bioactive agents have incorporated into the adhesive systems in an attempt to improve the durability of resin–dentin adhesive interfaces *in vitro*[3,9,23,24] and *in vivo*.[25,26] However, the interactions between resins monomers and drug particles can affect physicochemical properties of adhesive systems, such as monomer flexibility, degree of conversion, crosslinking density, hydrophilicity, and drug release.[10]

The adhesive systems should release enzymatic inhibitors in minimum concentrations to inactive MMPs and CTs. Epigallocatechin-3-gallate inhibited MMP-2 and MMP-9 at concentrations of 6 μM (≈20 μg/ml) and 0.8 μM (≈3 μg/ml), respectively[7,8], while CT-B were inhibited at 6,500 μg/ml.[4] In the present study, after 4320 h of epigallocatechin-3-gallate release in 1 mL of distilled water, all groups demonstrated rates cumulative release that could inhibit MMPs, but not CTs. However, the amount of water within the hybrid layer created by simplified adhesives is low, once that fluid flow rate is approximately 4 μL cm^{-2} min^{-1}.[27] Thus, it is possible that the concentration of EGCG released within the interface is higher than that presented in the current study, indicating a limitation of release assay.

When adhesive system was loaded directly with EGCG at 0.01% and 0.1% presented a low and constant EGCG release. Pallan *et al.*[10] observed similar release pattern using experimental resins incorporated with epigalocatechin-3-gallate at 0.2%, 1% and 2%. The low release of epigallocatechin-3-gallate may be of concern because MMPs with residual activity could continue degrading the collagen fibrils of the hybrid layer. However, Du *et al.*[9] demonstrated that direct incorporation of catechin at 0.01% to adhesive systems were able to maintain the bond strength up to 6 months, showing that maybe the low epigallocatechin-3-gallate release does not affect the bond strength.

In contrast, the adhesive systems incorporated with PLGA microparticles presented a pulsatile-release profiles. Poly (lactide-co-glycolide acid) is known in medical science as the best biomaterial available for controlled drug delivery in relation to design and performance.[16,28,29] Pulsatile release allows that higher doses are reached in consecutive moments,[30] which probably can be important to inactivate dentin endopeptidases that degrade the hybrid layer. After 4320 h of EGCG release, the majority of PLGA 75:25 groups presented cumulative release (%) significant lower than PLGA 50:50 groups ($p<0.05$). Therefore, the first null hypothesis was rejected.

Mechanical properties of the resin-based bonding systems are strongly depend upon the DC.[31] Epasinghe *et al*.[24] speculated that epigalocatechin-3-gallate may disturb the free radical polymerization of adhesive systems due to their free radical scavenging effect. Nevertheless, some studies demonstrated that the incorporation of epigalocatechin-3-gallate in low concentrations into adhesive systems did not cause any negative effect on the DC.[9-11] These facts corroborate with present study results. Probably, the catechin seems to have been entrapped within the linear chains after curing without interfering with monomers conversion.[11] In addition, PLGA microparticules did not interfere on the DC of two-step etch-and-rinse adhesive system. It is possible that DC was not affected by the addition of EGCG-loaded microparticles due to the existence of chemical compatibility between the resin monomers of adhesive system and the poly (lactide-co-glycolide) acid, once that both have polymeric nature.[16]

Epigallocatechin-3-gallate at 0.01% and 0.1% incorporated directly into adhesive systems did not affect the FS and E, corroborate with Neri *et al*.[11] On the other hand, when etch-and-rinse adhesive system was incorporated with EGCG loaded PLGA microparticules there was an increase in FS. Probably the undissolved particles may have acted as reinforcement leading to the higher values in FS.[32] Therefore, the second null hypothesis was rejected.

Polymers are considered insoluble structures that exhibit relatively high chemical stability.[33] Nevertheless, when exposed to oral environment the polymeric materials tend to begin a continuous process of water absorption and loss of soluble components, these known phenomena, respectively, sorption and solubility.[10,34] Polymer hydrophilicity is directly linked to its chemical composition.[33] Simplified adhesives systems, as Adper Single Bond 2, are composed of methacrylate monomers, which chemical structures contain polar groups (esters and hydroxyls groups) that having affinity for water.[33,34] Water molecules bind to polar groups through hydrogen bonding and breaks covalent bonds between the polymers, and consequently promotes the leaching of components of the adhesive system.[33,35] Some studies[36-38] related high sorption and solubility values to Adper Single Bond 2, corroborating with our results. Thus, it is plausible to believe that the incorporation of the hydrophilic drugs in the adhesive systems could result in an increase of sorption and solubility.[11] Epigallocatechin-3-galate is a polyphenol that have a greater hydrogen bonding capability due to the presence of three vicinal hydroxyl groups from the galloyl moiety.[4] However, incorporation of EGCG directly at 0.01% and 0.1% or in polymeric microparticules at 1% did not increase the water sorption of simplified adhesive system. One reason for WS results can be attributed chemical properties of poly (lactide-co-glycolide), since it is relatively hydrophobic and water insoluble,[39] so it would have

a limited ability to attract water into the polymer. In addition, Pallan *et al.*[10] showed that addition of epigallocatechin-3-gallate up to 2% did not affect WS of polymeric materials. It appears that low concentrations of this polyphenol was not capable of causing significant changes in the polarity of adhesive systems.[10,11]

One of the consequences of water sorption is the solubility phenomenon.[34,36] Solubility is the process characterized by leaching the material components of polymeric materials such solvents, unreact monomers and oligomers.[33] In the scientific literature polymeric materials doped directly with Epigallocatechin-3-gallate has shown conflicting solubility results. Pallan *et al.*[10] showed that the increase of solubility values of experimental resins by Epigallocatechi-3-gallate incorporation had concentration-dependent effect. However, when one-step self-etch adhesive system was doped with Epigallocatechin-3-gallate at 0.01% and 0.1% resulted in a decrease of solubility.[11] In the present study EGCG did not interfere in SL values, independent of concentration used and incorporation modes (directly or loaded PLGA microparticules) in two-step self-adhesive.

Immediate bond strength depends on the adequate resin monomers penetration into the interfibrillar spaces of the collagen web in the demineralized dentin, leading to the formation of the hybrid layer.[40] Nevertheless, differences in the composition of adhesive systems can influence in the bond strength of resin-dentin interface.[41] In the current study, the incorporation of epigallocatechin-3-gallate into the adhesive systems did not affect the resin-dentin bond strength after 24h corroborating with Du *et al.*[9] Epigallocatechin-3-gallate molecules interact with dentin collagen fibrils producing crosslinking and reduces the enzymatic activity of MMPs and CTs, which may be important to the longevity of the adhesive procedures.[4]

Although epigallocatechin-3-gallate incorporation in adhesive systems present promising results in sound dentin, it is necessary more long evaluation periods to confirm the maintenance of resin-dentin bond strength and further studies evaluating the catechin efficiency in the preservation of adhesive interfaces performed in affected caries dentin.

CONCLUSION

Single Bond 2 doped with EGCG-load PLGA 50:50 microparticules at 1% promote controlled release without causing detrimental effects of physicochemical properties.

ACKNOWLEDGEMENTS

This work was funded thanks to grants CNPq 140058/2013-3 and CNPq 140073/2014-0. FUNCAP CI1-0800-000540100/13

REFERENCES

1. Al-Ammar A, Drummond JL, Bedran-Russo AK. The use of collagen cross-linking agents to enhance dentin bond strength. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2009; 91: 419-24.
2. Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, Aguiar TR, Vidal CM, Napotilano JG, Nam JW, Leme AA. Dentin biomodification: strategies, renewable resources and clinical applications. *Dental Materials* 2014; 30: 62-76.
3. Santiago SL, Osorio R, Neri JR, Carvalho RM, Toledano M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. *Journal of Adhesive Dentistry* 2013; 15: 535-40.
4. Vidal CM, Aguiar TR, Phansalkar R, McAlpine JB, Napolitano JG, Chen SN, Araújo LS, Pauli GF, Bedran-Russo A. Galloyl moieties enhance the dentin biomodification potential of plant-derived catechins. *Acta Biomaterialia* 2014; 10: 3288-94.
5. Epasinghe DJ, Yiu CK, Burrow MF, Tsoi JK, Tay FR. Effect of flavonoids on the mechanical properties of demineralised dentine. *Journal of Dentistry* 2014; 42: 1178-84.
6. Devika PT, Prince PS. Preventive effect of (-)epigallocatechin-gallate (EGCG) on lysosomal enzymes in heart and subcellular fractions in isoproterenol-induced myocardial infarcted Wistar rats. *Chemico-Biological Interactions* 2008; 172: 245-52
7. Demeule M, Brossard M, Pagé M, Gingras D, Béliveau R. Matrix metalloproteinase inhibition by green tea catechins. *Biochimica et Biophysica Acta* 2000; 1478: 51-60.
8. Garbisa S, Sartor L, Biggin, S. Salvato, B, Benelli, R, Albini A. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. *Cancer* 2001; 91: 822-32.
9. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. *Journal of Dentistry* 2012; 40: 485-92.
10. Pallan S, Furtado Araujo MV, Cilli R, Prakki A. Mechanical properties and characteristics of developmental copolymers incorporating catechin or chlorhexidine. *Dental Materials* 2012; 28: 687-94.

11. Neri JR, Yamauti M, Feitosa VP, Pires AP, Araújo Rdos S, Santiago SL. Physicochemical Properties of a Methacrylate-Based Dental Adhesive Incorporated with Epigallocatechin-3-gallate. *Brazilian Dental Journal* 2014; 25: 528-31.
12. Chen H, Huang B. Effect of EGCG Application on Collagen Degradation in Dentine Caries. *Applied Mechanics and Materials* 2013; 455: 112-6.
13. Jackson JK, Zhao J, Wong W, Burt HM. The inhibition of collagenase induced degradation of collagen by the galloyl-containing polyphenols tannic acid, epigallocatechingallate and epicatechingallate. *Journal of Materials Science: Materials in Medicine* 2010; 21: 1435-43.
14. Matsumoto A, Matsukawa Y, Suzuki T, Yoshino H. Drug release characteristics of multi-reservoir type microspheres with poly(dl-lactide-co-glycolide) and poly(dl-lactide). *Journal of Controlled Release* 2005; 106: 172-80.
15. Li M, Rouaud O, Poncelet D. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. *International Journal of Pharmaceutics* 2008; 363: 26-39.
16. Makadia HK, Siegel SJ. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers (Basel)* 2011; 3: 1377-97.
17. Cappellano G, Woldetsadik AD, Orilieri E, Shivakumar Y, Rizzi M, Carniato F, Gigliotti CL, Boggio E, Clemente N, Comi C, Dianzani C, Boldorini R, Chiocchetti A, Renò F, Dianzani. Subcutaneous inverse vaccination with PLGA particles loaded with a MOG peptide and IL-10 decreases the severity of experimental autoimmune encephalomyelitis. *Vaccine* 2014; 32: 5681-9.
18. Gaignaux A, Réeff J, Siepmann F, Siepmann J, De Vriese C, Goole J, Amighi K. Development and evaluation of sustained-release clonidine-loaded PLGA microparticles. *International Journal of Pharmaceutics* 2012; 437: 20-8.
19. Sousa FF, Luzardo-Alvarez A, Pérez-Estévéz A, Seoane-Prado R, Blanco-Méndez J. Development of a novel AMX-loaded PLGA/zein microsphere for root canal disinfection. *Biomedical Materials* 2010; 5: 055008.
20. Gaglianone LA, Lima AF, Gonçalves LS, Cavalcanti AN, Aguiar FH, Marchi GM. Mechanical properties and degree of conversion of etch-and-rinse and self-etch adhesive systems cured by a quartz tungsten halogen lamp and a light-emitting diode. *Journal of the Mechanical Behavior of Biomedical Materials* 2012; 12: 139-43.

21. Collares FM, Ogliari FA, Zanchi CH, Petzhold CL, Piva E, Samuel SM. Influence of 2-hydroxyethyl methacrylate concentration on polymer network of adhesive resin. *Journal of Adhesive Dentistry* 2011; 13: 125-9.
22. Cadenaro M, Pashley DH, Marchesi G, Carrilho M, Antoniolli F, Mazzoni A, Tay FR, Di Lenarda R, Breschi L. Influence of chlorhexidine on the degree of conversion and E-modulus of experimental adhesive blends. *Dental Materials* 2009; 25: 1269-74.
23. Carrilho MR, Carvalho RM, de Goes MF, di Hipólito V, Geraldeli S, Tay FR, Pashley DH, Tjäderhane L. Chlorhexidine preserves dentin bond in vitro. *Journal of Dental Research* 2007; 86: 90-4.
24. Epasinghe DJ, Yiu CK, Burrow MF, Tay FR, King NM. Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength. *Journal Dentistry* 2012; 40: 173-80.
25. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, Pashley D. In vivo preservation of the hybrid layer by chlorhexidine. *Journal of Dental Research* 2007; 86: 529-33.
26. Ricci HA, Sanabe ME, de Souza Costa CA, Pashley DH, Hebling J. Chlorhexidine increases the longevity of in vivo resin-dentin bonds. *European Journal of Oral Sciences* 2010; 118:411-6.
27. Hashimoto M1, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding. *Journal of Dental Research*. 2004; 83:843-8.
28. Pan Q, Xu Q, Boylan NJ, Lamb NW, Emmert D, Yang JC, Tang L, Heflin T, Alwadani S, Eberhart CG, Stark WJ, Hanes J. Corticosteroid-loaded biodegradable nanoparticles for prevention of corneal allograft rejection in rats. *Journal of Controlled Release* 2015; 201: 32-40.
29. Silva AL, Rosalia RA, Varypataki E, Sibuea S, Ossendorp F, Jiskoot W. Poly-(lactic-co-glycolic-acid)-based particulate vaccines: Particle uptake by dendritic cells is a key parameter for immune activation. *Vaccine* 2015; doi: 10.1016/j.vaccine.2014.12.059. [Epub ahead of print]
30. Huang X, Brazel CS. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *Journal of Controlled Release* 2001; 73: 121-36.
31. Solhi L, Atai M, Nodehi A, Imani M. A novel dentin bonding system containing poly(methacrylic acid) grafted nanoclay: synthesis, characterization and properties. *Dental Materials* 2012; 28: 1041-50.

32. K.J. Anusavice, N.Z. Zhang, C. Shen. Controlled release of chlorhexidine from UDMA-TEGDMA resin. *Journal of Dental Research* 2006; 85: 950-4.
33. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. *Dental Materials* 2006; 22: 211-22.
34. Merdas I, Tcharkhtchi A, Thominette F, Verdu J, Dean K, Cook K. Water absorption by uncrosslinked polymers, networks and IPNs having medium to high polarity. *Polymer* 2002; 43: 4619-25.
35. Salz U, Zimmermann J, Zeuner F, Moszner N. Hydrolytic stability of self-etching adhesive systems. *Journal of Adhesive Dentistry* 2005; 7:107-16.
36. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, Carrilho MR. Water sorption/solubility of dental adhesive resins. *Dental Materials* 2006; 22: 973-80.
37. Wambier L, Malaquias T, Wambier DS, Patzlaff RT, Bauer J, Loguercio AD, Reis A. Effects of prolonged light exposure times on water sorption, solubility and cross-linking density of simplified etch-and-rinse adhesives. *Journal of Adhesive Dentistry* 2014; 16:229-34.
38. Fabre HS, Fabre S, Cefaly DF, de Oliveira Carrilho MR, Garcia FC, Wang L. Water sorption and solubility of dentin bonding agents light-cured with different light sources. *Journal of Dentistry* 2007; 35: 253-8.
39. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. *Journal of Controlled Release* 2012; 161: 505-22.
40. Hashimoto M, Nagano F, Endo K, Ohno H. A review: Biodegradation of resin–dentin bonds. *Japanese Dental Science Review* 2011; 47: 5-12
41. Zanchi CH1, Münchow EA, Ogliari FA, Chersoni S, Prati C, Demarco FF, Piva E. Development of experimental HEMA-free three-step adhesive system. *Journal of Dentistry* 2010; 38:503-8.

TABLES

Table 1 – Adhesive system and bonding procedures

Product	Composition	Manufacturer (#Batch nº)	Application mode
Adper Single Bond 2	Adhesive–Bis-GMA, HEMA, dimethacrylates, silica nanofiller (5 nm), polyalquenoic acid copolymer, initiators, water and ethanol	3M ESPE, St.Paul, MN, USA (batch #1312201025)	1-two coats of adhesive; 2-air-drying for 10 s at 20 cm; 3-light-curing for 10 s.

Abreviations: Bis-GMA: bisphenol A diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate. *This brand name is the same product as Adper Scotchbond 1 XT, Adper Single Bond Plus and Adper Single Bond 1 XT.

Table 2.Mean (standard deviation) of physicochemical properties tested.

Groups (*n*=10)	Degree of conversion %	Flexural Strength (MPa)	Elastic Modulus (GPa)	Water Sorption (mg/mm^3)	Solubility ($\mu g/mm^3$)
SB (Control)	59.8 (2.0)	12.2 (3.1)[a]	0.2 (0.06)	172.7 (7.2)	73.4 (5.8)
EGCG 0.01%	58.3 (3.3)	12.4 (2.7)[a]	0.2 (0.1)	175.5 (6.3)	74.7 (2.8)
EGCG 0.1%	59.0 (3.6)	11.9 (3.7)[a]	0.2 (0.09)	173.6 (9.6)	76.3 (5.2)
1% PLGA50:50/EGCG	58.7 (2.8)	16.5 (3.4)[b]	0.2 (0.04)	166.9 (7.4)	77.0 (4.3)

*Distinct superscript letters indicate statistical difference in the same columns ($p<0.05$).

Table 3 – Bond strength values (MPa ± SD (*)) according to adhesive systems used.

Groups (*n*=9)	Adper Single Bond 2
	24 hours (ns)
SB	34.6 ± 6.9 (47)
EGCG 0.01%	36.6 ± 5.7 (53)
EGCG 0.1%	38.6 ± 4.7 (52)
1% PLGA50:50/EGCG	35.1 ± 6.7 (40)

ns: no statistical significance between values (p>0.05).

(*) corresponds to the number of sticks tested per group.

Table 4 – Distribution of mode of fracture of each group expressed as *n* (relative percentage)

Groups	Adper Single Bond 2				
	24 hours				
	A	M	CR	CD	PF
SB	7 (14)	40 (78)	1 (2)	2 (4)	1(2)
EGCG 0.01%	1 (2)	52 (88)	3 (5)	1 (2)	2(3)
EGCG 0.1%	3 (5)	49 (82)	3 (5)	3 (5)	2(3)
1% PLGA50:50/EGCG	2 (4)	38 (81)	3 (7)	2 (4)	2(4)

A: adhesive failure; M: mixed failure; CR: cohesive failure in resin; CD: cohesive failure in dentin; PF: premature failure

FIGURES

Incorporation mode of EGCG	Adhesive system	Description
-	SB (Control)	-
Directly	SB + EGCG 0.01% (EGCG 0.01%)	Containing 0.01% of EGCG
	SB+EGCG 0.1%	Containing 0.1% of EGCG
Microencapsulated	SB+0.5% PLGA50:50/EGCG	Containing 0.5% (w/w) of microparticles (PLGA50:50) loaded with EGCG (≈ 0.02% EGCG)
	SB+1% PLGA50:50/EGCG	Containing 1.0% (w/w) of microparticles (PLGA50:50) loaded with EGCG (≈ 0.04% EGCG)
	SB+2% PLGA50:50/EGCG	Containing 2.0% (w/w) of microparticles (PLGA50:50) loaded with EGCG (≈ 0.08% EGCG)
	0.5% PLGA75:25/EGCG	Containing 0.5% (w/w) of microparticles (PLGA75:25) loaded with EGCG (≈ 0.02% EGCG)
	SB+1% PLGA75:25/EGCG	Containing 1.0% (w/w) of microparticles (PLGA75:25) loaded with EGCG (≈ 0.04% EGCG)
	SB+2% PLGA75:25/EGCG	Containing 2.0% (w/w) of microparticles (PLGA75:25) loaded with EGCG (≈ 0.08% EGCG)

Figure 1 – Experimental adhesives systems

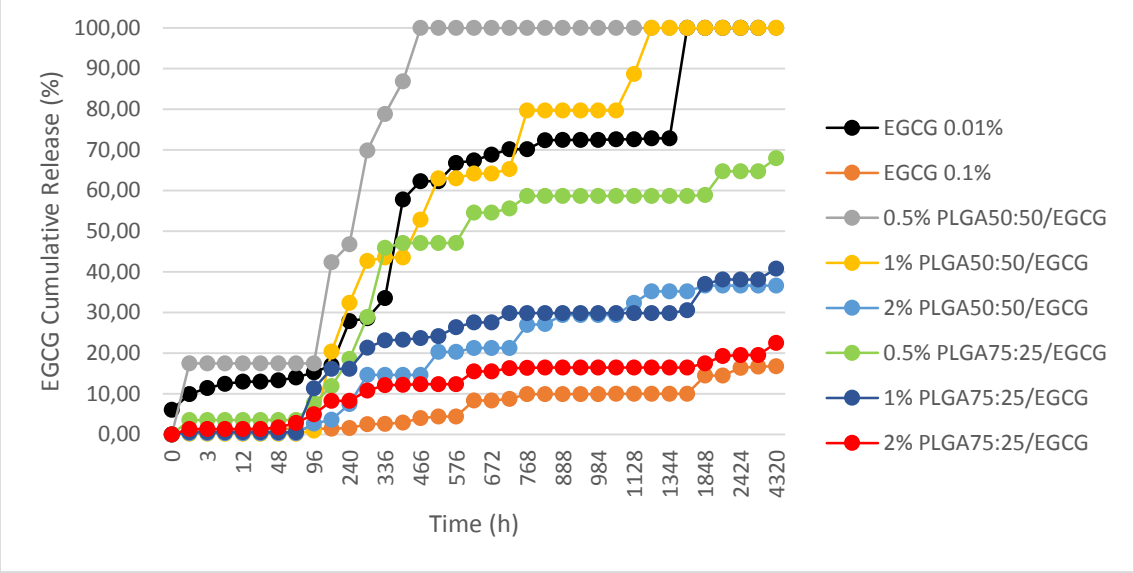


Figure 2. EGCG cumulative release (%) from adhesive systems during the entire evaluation period (4320 hours).

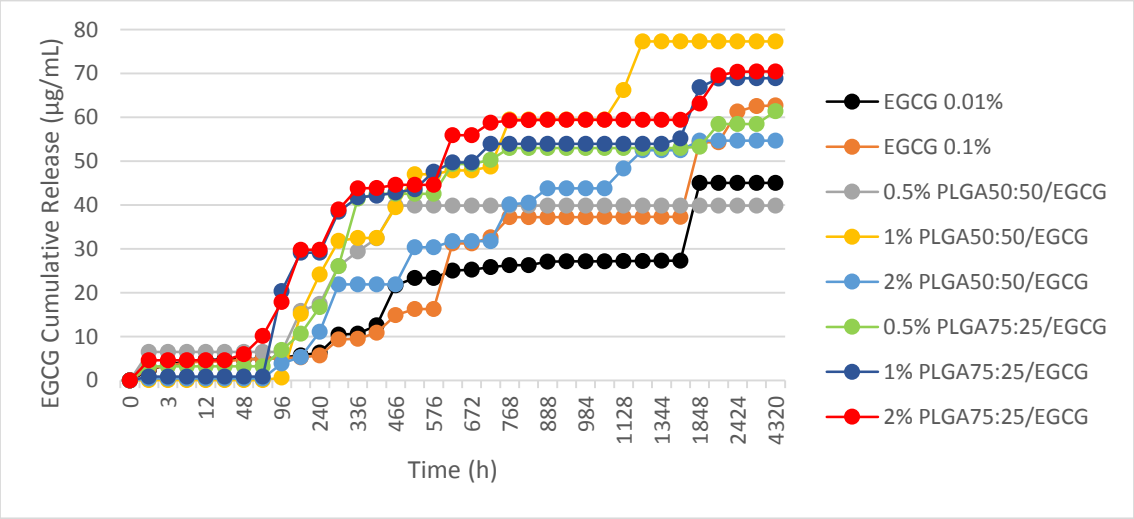


Figure 3. EGCG cumulative release (µg/mL) from adhesive systems during the entire evaluation period (4320 hours).

Conclusão Geral

4 CONCLUSÃO GERAL

Diante dos resultados obtidos no presente estudo e considerando as condições experimentais empregadas, pode-se concluir que:

1) O pré-tratamento dentinário com solução ácida de fluoreto de sódio (pH 3,6) pode ser uma alternativa viável para melhorar a longevidade dos procedimentos adesivos executados com sistemas adesivos autocondicionantes de 2 passos;
2) A biomodificação dentinária, com o flavonóide epigalocatequina-3-galato e o digluconato de clorexidina, é eficiente em manter a resistência de união de sistema adesivo autocondicionante de passo único ao longo de 6 e 12 meses;
3) A incorporação de epigalocatequina-3-galato, em baixas concentrações (0,01% e 0,1%), em um sistema adesivo autocondicionante de passo único reduz a solubilização dos seus componentes. Contudo, o epigalocatequina-3-galato não interfere significativamente nos valores de sorção, grau de conversão e resistência flexural;
4) Micropartículas de ácido polilático glicólico carregadas com epigalocatequina-3-galato, especialmente formuladas com PLGA 50:50, poderiam ser uma ferramenta útil para a liberação controlada de fármacos.
5) O Single Bond 2 incorporado com micropartículas de PLGA 50:50 carregadas com EGCG a 1% promove liberação controlada da droga sem prejudicar as propriedades físico químicas do sistema adesivo.

Portanto, conclui-se que as diferentes abordagens de biomodificação dentinária, de uma forma geral, além de não prejudicarem as propriedades físicas dos sistemas adesivos estudados, podem também proporcionar benefícios às interfaces de união, podendo mantê-las mais estáveis ao longo do tempo.

Referências

REFERÊNCIAS

AL-AMMAR, A.; DRUMMOND, J. L.; BEDRAN-RUSSO, A. K. The use of collagen cross-linking agents to enhance dentin bond strength. **Journal of Biomedical Materials Research Part B: Applied Biomaterials**, v. 91, p. 419-424, 2009.

ANDERSON, J. M.; SHIVE, M. S. Biodegradation and biocompatibility of PLA and PLGA microspheres. **Advanced Drug Delivery Reviews**, v. 28, p. 5-24, 1997.

BEDRAN-RUSSO, A.K *et al*. Dentin biomodification: strategies, renewable resources and clinical applications. **Dental Materials**, v. 30, p. 62-76, 2014.

BRESCHI, L. *et al*. Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: A 12-month in vitro study. **Journal of Adhesive Dentistry**, v. 11, p. 191-198, 2009.

BRESCHI, L. *et al*. Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. **Dental Materials**, v. 26, p. 320-325, 2010.

BRESCHI, L. *et al*. Dental adhesion review: Aging and stability of the bonded interface. **Dental Materials**, v. 24, p. 90-101, 2008.

BRÖMME, D.; OKAMOTO, K. Human cathepsin O2, a novel cysteine protease highly expressed in osteoclastomas and ovary molecular cloning, sequencing and tissue distribution. **Biological Chemistry Hoppe Seyler**, v. 376, p. 379-384, 1995.

CAMPOS, E. A. *et al*. Influence of chlorhexidine concentration on microtensile bond strength of contemporary adhesive systems. **Brazilian Oral Research**, v. 23, p. 340-345, 2009.

CAO, Y.; CAO, R. Angiogenesis inhibited by drinking tea. **Nature**, v. 398, p. 381, 1999.

CARDOSO, M.V. *et al*. Current aspects on bonding effectiveness and stability in adhesive dentistry. **Australian Dental Journal**, n. 56 (supl 1), v. 31-44, 2011.

CARRILHO, M. R. *et al*. Chlorhexidine preserves dentin bond in vitro. **Journal of Dental Research**, v. 86, p. 90-94, 2007a.

CARRILHO, M. R. *et al*. Chlorhexidine preserves dentin bond in vitro. **Journal of Dental Research**, v. 86, p. 529-533, 2007b.

DELL'AICA, I. *et al*. Matrix proteases, green tea, and St. John's wort: Biomedical research catches up with folk medicine. **Clinica Chimica Acta**, v. 38, p. 38:69-77, 2007.

DEMEULE, M. *et al*. Matrix metalloproteinase inhibition by green tea catechins. **Biochimica et Biophysica Acta**, v. 1478, p. 51-60, 2000.

DE MUNCK, J. *et al*. A Critical Rewiew of the durability of adhesion to tooth tissue: Methods and results. **Journal of Dental Research** v. 84, v. 2, p. 118-132, 2005.

DE SOUZA, L. B. *et al*. Cytotoxic effects of different concentrations of chlorhexidine. **American Journal of Dentistry**, v. 20, p. 400-404, 2007.

DEVIKA, P. T.; PRINCE, P. S. Preventive effect of (-)epigallocatechin-gallate (EGCG) on lysosomal enzymes in heart and subcellular fractions in isoproterenol-induced myocardial infarcted Wistar rats. **Chemico-Biological Interactions**, v. 172, p. 245-252, 2008.

DU, X. *et al*. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. **Journal of Dentistry**, v. 40, p. 485-492, 2012.

FARIA, G. *et al*. Evaluation of chlorhexidine toxicity injected in the paw of mice and added to cultured l929 fibroblasts. **Journal of Endodontics**, v. 33, p. 715-722, 2007.

GAIGNAUX, A. *et al*. Development and evaluation of sustained-release clonidine-loaded PLGA microparticles. **International Journal of Pharmaceutics**, v. 437, p. 20-28, 2012.

GARBEROGLIO, R.; BRÄNNSTRÖM, M. Scanning electron microscopic investigation of human dentinal tubules. **Archives of Oral Biology**, v. 21, p. 355-362, 1976.

GARBISA, S. *et al*. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. **Cancer**, v. 91, p. 822-832, 2001.

GENDRON, R. *et al*. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. **Clinical and Diagnostic Laboratory Immunology**, v. 6, p. 437-439, 1999.

HEBLING, J. *et al*. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. **Journal of Dental Research**, v. 84, p. 741-746, 2005.

HOU, W. S. *et al*. Comparison of cathepsins K and S expression within the rheumatoid and osteoarthritic synovium. **Arthritis & Rheumatology**, v. 46, p. 663-674,. 2002.

ITOTA, T. *et al*. Effect of fluoride application on tensile bond strength of self-etching adhesive systems to demineralized dentin. **Journal of Prosthetic Dentistry**, v. 88, p. 503-510, 2002.

JACKSON, J. K. *et al*. The inhibition of collagenase induced degradation of collagen by the galloyl-containing polyphenols tannic acid, epigallocatechin gallate and epicatechin gallate. **Journal of Materials Science: Materials in Medicine**, v. 21, p.1435-1443, 2010.

JAIN, R. A. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. **Biomaterials**, v. 21, p. 2475-2490, 2000.

KATO, M. T. *et al*. Sodium Fluoride Inhibits MMP-2 and MMP-9. **Journal of Dental Research**, v. 93, p. 74-77, 2014.

LI, M.; ROUAUD, O.; PONCELET, D. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. **International Journal of Pharmaceutics**, v. 363, p. 26-39, 2008.

LI, Z. *et al*. Collagenase activity of cathepsin K depends on complex formation with chondroitin sulfate. **The Journal of Biological Chemistry**, v. 277, p. 28669-28676, 2002.

LIANG, H. F. *et al*. Preparation of nanoparticles composed of poly(gamma-glutamic acid)-poly(lactide) block copolymers and evaluation of their uptake by HepG2 cells. **Journal of Controlled Release**, v. 105, p.:213-225, 2005.

LOGUERCIO, A. D. *et al*. Influence of chlorhexidine digluconate concentration and application time on resin–dentin bond strength durability. **European Journal of Oral Sciences**, v. 117, p. 587-596, 2009.

MARSHALL, G. W. JR. Dentin: microstructure and characterization **Quintessence International**, v. 24, p. 606-617, 1993.

MARSHALL, G. W. JR. *et al*. The dentin substrate: structure and properties related to bonding. **Journal of Dentistry**, v. 25, p. 441-458, 1997.

MATSUMOTO, A. *et al*. Drug release characteristics of multi-reservoir type microspheres with poly(dl-lactide-co-glycolide) and poly(dl-lactide). **Journal of Controlled Release**, v. 106, p. 172-180, 2005.

MAZZONI, A. *et al*. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. **Journal of Dental Research**, v. 92, p. 82-86, 2013.

MAZZONI, A. *et al*. Role of Dentin MMPs in Caries Progression and Bond Stability. **Journal of Dental Research**, v. 94, p. 241-251, 2015.

MEI, M. L. *et al*. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. **Dental Materials**, v. 28, p. 903-908, 2012.

NAGASE, H.; WOESSNER, J. F. JR. Matrix metalloproteinases. **Journal of Biological Chemistry**, v. 274, p. 21491-21494, 1999.

NASCIMENTO, F. D. *et al*. Cysteine cathepsins in human carious dentin. **Journal of Dental Research**, v. 90, p. 506-511, 2011.

NERI, J. R. *et al*. Efficacy of smear layer removal by cavity cleaning solutions: an atomic force microscopy study. **Journal of Dental Sciences**, v. 26, p. 253-257, 2011.

NISHITANI, Y. *et al*. Activation of gelatinolytic/ collagenolytic activity in dentin by self-etching adhesives. **European Journal of Oral Sciences**, v. 114, p. 160-166, 2006.

OSORIO, R. *et al*. Zinc-doped dentin adhesive for collagen protection at the hybrid layer. **European Journal of Oral Sciences**, v. 119, p. 401-410, 2011.

OSORIO, R. *et al*. MMPs activity and bond strength in deciduous dentine-resin bonded interfaces. **Journal of Dentistry**, v. 41, p. 549-555, 2013.

PALLAN, S. *et al*. Mechanical properties and characteristics of developmental copolymers incorporating catechin or chlorhexidine. **Dental Materials**, v. 28, p. 687-694, 2012.

PASHLEY, D. H.; CARVALHO, R. M. Dentine permeability and dentine adhesive. **Journal of Dentistry**, v. 25, p. 355-372, 1997.

PASHLEY, D. H. *et al*. Collagen degradation by host-derived enzymes during aging. **Journal of Dental Research**, v. 83, p. 216-221, 2004.

SANTIAGO S. L. *et al*. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. **Journal of Adhesive Dentistry**, v. 15, p. 535-540, 2013.

SCAFFA, P. M. *et al*. Chlorhexidine inhibits the activity of dental cysteine cathepsins. **Journal of Dental Research**, v. 91, p. 420-425, 2012.

STANISLAWCZUK, R. *et al*. Chlorhexidine-containing acid conditioner preserves the Longevity of resin-dentin bonds. **Operative Dentistry**, v. 34, p. 481-490, 2009.

SEN, T. *et al*. Multifunctional effect of epigallocatechin-3-gallate (EGCG) in downregulation of gelatinase-A (MMP-2) in human breast cancer cell line MCF-7. **Life Sciences**, v. 84, p. 194-204, 2009.

TAMBER, H. *et al*. Formulation aspects of biodegradable polymeric microspheres for antigen delivery. **Advanced Drug Delivery Reviews**, v. 10, p. 357-376, 2005.

TAY, F. R.; PASHLEY, D. Water treeing--a potential mechanism for degradation of dentin adhesives. **American Journal of Dentistry**, v. 16, p. 6-12, 2003.

TENUTA, L. M.; CURY, J. A. Fluoride: its role in dentistry. **Brazilian Oral Research**, v. 24 (supl 1), p. 9-17, 2010.

TENUTA, L. M. *et al*. Fluoride release from CaF2 and enamel demineralization. **Journal of Dental Research**, v. 87, p. 1032-1036, 2008.

TERSARIOL, I. L. *et al*. Cysteine cathepsins in human dentin-pulp complex. **Journal of Endodontics**, v. 36, p. 475-481, 2010.

TJÄDERHANE, L. *et al*. Dentin basic structure and composition – an overview. **Endodontic Topics**, v. 20, p. 3-29, 2012.

TJÄDERHANE, L. *et al*. The activation and function of host matrix metalloproteinase in dentin matrix during breakdown in carious lesions. **Journal of Dental Research**, v. 77, p. 1622-1629, 1998.

TJÄDERHANE, L. *et al*. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. **Dental Materials**, v. 29, p. 116-135, 2013a.

TJÄDERHANE, L. *et al*. Strategies to prevent hydrolytic degradation of the hybrid layer-A review. **Dental Materials**, v. 29, p. 999-1011, 2013b.

TOLEDANO, M. *et al*. A Zn-doped etch-and-rinse adhesive may improve the mechanical properties and the integrity at the bonded-dentin interface. **Dental Materials**, v. 29, p. 142-152, 2013.

TOLEDANO, M. *et al*. Zinc-inhibited MMP-mediated collagen degradation after different dentine demineralization procedures. **Caries Research**, v. 46, p. 201-207, 2012.

TURK, V. *et al*. Cysteine cathepsins: from structure, function and regulation to new frontiers. **Biochim Biophys Acta**, v. 1842, p. 68-88, 2012.

VAN MEERBEEK, B. *et al*. State of the art of self-etch adhesives. **Dental Materials**, v. 27, p. 17-28, 2011.

VAN STRIJP, A. J. *et al*. Host-derived proteinases and degradation of dentine collagen in situ. **Caries Research**, v. 37, p. 58-65, 2003.

VIDAL, C. M. *et al*. Galloyl moieties enhance the dentin biomodification potential of plant-derived catechins. **Acta Biomaterialia**, v. 10, p3288-3294, 2014a.

VIDAL, C. M. *et al*. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. **Journal of Dental Research**, v. 93, p. 269-274, 2014b.

VISSE, R.; NAGASE, H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases. Structure, function, and biochemistry. **Circulation Research**, v. 92, p. 827-839, 2003.

ZHOU, J. *et al*. MMP-inhibitory effect of chlorhexidine applied in a self-etching adhesive. **Journal of Adhesive Dentistry**, v. 13, p. 111-115, 2011.

Anexos

ANEXO A – Instruções para os autores do periódico "*European Journal of Oral Siences*", referente ao Capítulo 1.

Author Guidelines

European Journal of Oral Siences
Information for Contributors

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

Useful Websites: Submission Site, Articles published in The European Journal of Oral Sciences, Author Services, Wiley-Blackwell's Ethical Guidelines, Guidelines for Biochemical/Biophysical Units & Nomenclature, Guidelines for Figures

1. GENERAL

The European Journal of Oral Sciences is an international non-profit journal which publishes original research papers within clinical dentistry, on all basic science aspects of structure, chemistry, developmental biology, physiology and pathology of relevant tissues, as well as on microbiology, biomaterials, and the behavioral sciences as they relate to dentistry. In general, analytical studies with a scientific novelty value are preferred to descriptive ones. Reviews, Focus Articles, Short Communications and Letters to the Editor will also be considered for publication.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards, as well as information concerning the procedure after a manuscript has been accepted for publication in the European Journal of Oral Sciences. Authors

are encouraged to visit Wiley-Blackwell Author Services for further information on the preparation and submission of articles and figures.

It is expected that all manuscripts submitted to the European Journal of Oral Sciences should follow journal format as described in the Author Guidelines and as displayed in recent issues of the Journal. Failure to do so reflects negatively on the work itself and may be a cause for immediate rejection of a manuscript.

2. ETHICAL GUIDELINES

The European Journal of Oral Sciences adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors, and that all authors agree to the submission of the manuscript to the Journal.

The European Journal of Oral Sciences adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned in the Acknowledgements.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles.

Animal experiments should be carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section that the study has been independently reviewed and approved by an ethical board, identifying the review and ethics committee for each study. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

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

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

2.4 DNA Sequences and Materials Requests

If a manuscript describes original nucleotide/amino acid sequence data, these should be submitted to GenBank by the authors and the accession numbers included in the manuscript. Authors of papers published in the Journal are obliged to honor any reasonable request by qualified investigators for unique propagative materials, such as cell lines, hybridomas, DNA clones and antibodies that are described in the paper.

2.5 Conflict of Interest

Authors are required to disclose any possible conflict of interest. These include financial issues (for example patent, ownership, stock ownership, consultancies, speaker's fee). Author's conflict of interest should be included under Acknowledgements.

2.6 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

2.7 Copyright Transfer Agreement

Upon acceptance of a manuscript, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services, where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper. Your article cannot be published until this has been done.

2.8 OnlineOpen

If the OnlineOpen option is selected, the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA): Creative Commons

3. MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site linked through the journal home page. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequently speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available online and below.

3.1. Getting Started

• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Firefox 1.0.4 or higher or Safari 1.2.4) and go to the journal home page. Click on 'Submit an Article'.

• Log-in or, if you are a new user click on 'register here'.

• If you are registering as a new user.

- After clicking on “Register here”, enter your name and e-mail information and click “Next”. Your e-mail information is very important.

- Enter your institution and address information as appropriate, and then click “Next.”

- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your area of expertise. Click “Finish”.

• The Journal strongly advises the use of professional mail and e-mail addresses rather than residential ones, both in accounts as well as in manuscripts.

• If you are registered, but have forgotten your log in details, enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.

• Log in and select “Corresponding Author Center”.

3.2. Submitting Your Manuscript

• After you have logged in to your 'Corresponding Author Center', you may submit a manuscript by clicking the submission link under 'Author Resources'.

• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript, and you may upload your pre-prepared covering letter.

• Click the “Next” button on each screen to save your work and advance to the next screen.

• You are required to upload your files.

- Click on the “Browse” button and locate the file on your computer.

- Select the designation of each file in the drop down next to the Browse button.

- When you have selected all files you wish to upload, click the “Upload Files” button.

• Be sure to upload a complete manuscript with all pages and sections as specified under 5.2 (below). It is of importance that a manuscript is adapted to journal format.

• Before uploading a manuscript, you must turn off Word’s automatic function for tracking of changes in the text. The uploaded manuscript should not display any track-changes.

• Review your submission (in HTML and PDF format) before completing your submission by sending to the Journal. Click the 'Submit' button when you are finished reviewing.

3.3. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected). Illustrations/Figures should be uploaded separately as TIFF, EPS, GIF, JPEG, PICT or Bitmap files. Do not embed illustrations in a .doc file and do not use PowerPoint. However, only high-resolution TIFF or EPS files are suitable for printing if the manuscript is accepted for publication. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the entire manuscript including title page, abstract page, text, references, tables, and figure legends, but no embedded figures. In the text, please reference any figures as “Figure 1”, “Figure 2” etc to match the Tag name you choose for all individual figure files uploaded. Tables may also be uploaded separately. Manuscripts should be formatted as described below. Please note that any manuscripts uploaded as Word 2007 (.docx) will be automatically rejected, implying that any .docx file should be saved as .doc before uploading.

3.4. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to make the final submission later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.5. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mail should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.6. Editorial Processing

After a first editorial screening, manuscripts will be forwarded to one of the Journal's Editors for further scientific evaluation and processing. Thus, queries and comments concerning a specific manuscript should primarily be directed to the managing Editor. Manuscripts submitted to the European Journal of Oral Sciences will be reviewed by two or more experts in the field. The European Journal of Oral Sciences uses single blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper.

3.7. Manuscript Status

You can access ScholarOne Manuscripts (formerly known as Manuscript Central) any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.8. Submission of Revised Manuscripts

To upload a revised manuscript, please locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision'. You should be careful not to upload the revised version under a new manuscript number as if it were another article. Be sure to use the earlier

manuscript number (which will then get an R addendum). Please remember to delete any old files uploaded when you upload your revised manuscript. Do not forget to submit an accompanying letter with itemized answers to all questions and remarks made by the reviewers and the Editor.

4. MANUSCRIPT TYPES ACCEPTED

Original Articles: An original article should comprise a conclusive, full-length scientific investigation. It should describe the rationale behind the study, the materials and methods used, and the results obtained. There should also be a discussion of the implications of the results as well as a list of literature references cited.

Scientific studies investigate phenomena and acquire new knowledge – or correct or integrate previous knowledge. They are based on the collection of data through observation and experimentation, and subject to specific principles of reasoning. The European Journal of Oral Sciences gives priority to analytical articles, investigating why and how something occurred rather than reporting empirical observations.

Review Articles: May be invited by the Editors. Proposals for such articles should be discussed with the appropriate Editor prior to preparation and submission. Review articles comprise attempts to synthesize the existing literature pertaining to a specific scientific question using methods and principles of reasoning that are as transparent as possible. It follows that systematic reviews are preferred over more narrative reviews. Review articles will be subjected to peer review.

Focus Articles: May be invited by the Editors. Proposals for such articles should be discussed with the appropriate Editor prior to preparation and submission. Focus articles may build on the same principles as the Review article, but are usually shorter and aim at stimulating a broader scientific discussion by ‘contesting conventional wisdom’ and allowing the author(s) to argue a specific point pertaining to a matter of current scientific importance. Focus articles will be subjected to peer review.

Short Communications: Short communications should aim at being no longer than two printed pages. They should contain important, new, definitive information of sufficient significance to warrant publication. Short communications need not follow the usual division into Material and methods etc. but should have a short Abstract.

Extra issues: Congress proceedings, larger papers or monographs may be published as Supplements or Part II issues, the full cost being paid by the congress organizer or similar. A condition is that the proposed extra issue is deemed to have a significant scientific value. In some cases, the Journal will partly fund extra issues; this is at the discretion of the Editor-in-Chief. Further information may be obtained from the Editor-in-Chief.

5. MANUSCRIPT FORMAT AND STRUCTURE

It is expected that all manuscripts submitted to the European Journal of Oral Sciences should follow journal format as described in the Author Guidelines and as displayed in recent issues of the Journal. Failure to do so reflects negatively on the work itself and may be a cause for immediate revision or even rejection of a manuscript.

5.1. Format

Language: The language of publication is English. Authors whose native language is not English are strongly advised to obtain assistance from someone proficient in scientific English. Manuscripts not submitted in the proper format or in poor English may be returned without review. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Abbreviations, Symbols and Nomenclature: Correct unit abbreviations should be used. Examples include ‘‘yr’’, ‘‘wk’’, ‘‘d’’, ‘‘h’’, ‘‘min’’, ‘‘s’’ and ‘‘μm’’ rather than ‘‘years’’, ‘‘weeks’’, ‘‘days’’, ‘‘hrs’’, ‘‘minutes’’, ‘‘sec’’ and ‘‘μ’’, respectively. For abbreviations of physical and chemical units and symbols, designation of isotopically labelled compounds, abbreviations which may be used without definition etc., the Biochemical Journal web site is a valuable resource. Scientific names of bacteria, binomials in italics, must be given in full when first mentioned. Subsequent mention may abbreviate genus, taking care that this abbreviation is unambiguous (Staph. or Strep. instead of S.).

5.2. Structure

All manuscripts submitted to the European Journal of Oral Sciences should include: Title page, Abstract Page, Introduction, Material and Methods, Results, Discussion, Acknowledgments, References, Figure Legends, Tables, and Figures, arranged in that order.

Authors are urged to consult a recent issue of the Journal to be familiar with style and format. The whole manuscript should be double-spaced, paginated, and submitted in correct English. The beginning of paragraphs should be properly marked with an indent. Avoid end-of-line hyphens.

Title Page: The title page should contain the following information in the order given: 1) the article title; 2) authors’ full names without degrees or titles; 3) authors’ institutional affiliations including city and country; 4) a running title, not exceeding 40 letters and spaces; 5) name, address, telephone, telefax and e-mail address of the author responsible for correspondence. The title should be concise but informative, include animal species used (if appropriate) and should not include any non-standard acronyms or abbreviations. The Journal does not favour titles of an affirmative character.

Abstract: A separate abstract page should contain the following: 1) authors’ surnames and initials; 2) title of manuscript; 3) the abbreviation Eur J Oral Sci; 4) the word Abstract followed by a summary of the complete manuscript; 5) up to five key words according to Index Medicus;

6) name, address, telefax and e-mail address of the author to whom requests for reprints should be sent. This contact information should refer to a professional rather than to a residential/private address.

The Abstract should give a condensed overview of the study, summarizing its background, aim, methodology and results with only few but relevant details, and the authors' principal conclusions. It should be short and concise, without headings and not divided into paragraphs, and with a maximum of 200 words. It should not contain any non-standard acronyms or abbreviations.

5.3 Main Text of Original Articles

Material and Methods: Procedures should be described in such a detail as to make it possible to repeat the work. Subheadings may be used to improve clarity.

It is assumed that authors have considered the ethical aspects of their research and ensured that the work was approved by an appropriate Ethical Committee. This should be stated. In human experimentation, informed consent from individuals must have been given. (See above under 2.2)

Sources of supply of commercial products should be given with the address (town, state and country) in parenthesis.

For an improved quality and transparency, reports of randomized trials must conform to the CONSORT guidelines and will be evaluated in light of the recommendations in this statement. (See above under 2.3)

Since many investigations rely on statistical treatment, authors are advised to consult a person with in-depth statistical knowledge.

If a manuscript describes original nucleotide/amino acid sequence data, these should be submitted to GenBank by the authors and the accession numbers included in the manuscript. (See above under 2.4)

Authors of papers published in the Journal are obliged to honor any reasonable request by qualified investigators for unique propagative materials, such as cell lines, hybridomas, DNA clones and antibodies that are described in the paper.

Results and Discussion: The Results section should clearly and concisely report findings, as a rule in the past tense, without subjective comments and reference to previous literature. Double documentation of data in text, tables or figures is not acceptable. Tables/figures should not include data that can be given in the text in one or two sentences. The Discussion section presents the interpretation of the findings; this is the only proper section for subjective comments. Authors are strongly urged to avoid undue repetition of what has already been reported in Results. For the sake of clarity, the Results section may have subheadings; this is usually not the case with the Discussion.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. This may include recognition of e.g. financial support, gifts of research material, assistance with statistics and language. Please also include specifications of any potential conflict of interests if appropriate.

Short Communications need not follow the usual division into Material and methods etc. but should have a short abstract.

Review and Focus Articles should include a Title page, an Abstract page and a Reference list as regular Original Research Articles. Although a Review article (particularly following a systematic review) may adhere to the format of the Original Research Article, Review and Focus articles need not contain Materials and Methods, Results or Discussion sections, and may instead employ other headings as relevant for the topic addressed.

5.4. References

Number references consecutively in the order in which they are first mentioned in the text. Identify references in texts, tables, and legends by Arabic numerals (within parenthesis). Check to ensure that all listed references are cited in the text. If an author's name is mentioned in the text, small capital letters should be used.

Non-refereed material and, if possible, non-English publications should be avoided. Congress abstracts, unaccepted papers, unpublished observations, and personal communications may not be placed in the Reference list. References to 'unpublished findings' and to 'personal communication' (provided explicit consent has been given by the sources) may be inserted in parentheses in the text. Unpublished articles should be referred to only if proof can be given that they are accepted for publication. Copies of such articles may be requested for evaluation of the manuscript submitted.

Authors are urged to study the examples of correct reference formats given below. For abbreviations of journals, consult the List of the Journals Indexed in Index Medicus. List all authors; do not use et al. in the Reference list. Avoid issue numbers in journal articles. Give first and last page of references in full.

Journals

Standard journal article:

JERNVALL J, THESLEFF I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 2000; 92: 19–29.

Article in supplement or special issue:

MUNDY GR. Cellular and molecular regulation of bone turnover. Bone 1999; 24 (Suppl): 35S–38S.

Corporate (collective) author:

WHO COLLABORATING CENTRE FOR ORAL PRECANCEROUS LESIONS. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978; 46: 518–539.

Unpublished article:

FLEISCHMANNOVA J, MATALOVA E, TUCKER AS, SHARPE PT. Mouse models of tooth abnormalities. Eur J Oral Sci 2008; 116: in press.

Books and other monographs:

Personal author(s):

PINDBORG JJ. Atlas of diseases of the oral mucosa, 5th ed. Copenhagen: Munksgaard, 1992; 50–66.

Chapter in book:

RUCH JV. Tooth morphogenesis and differentiation. In: LINDE A, ed. Dentin and dentinogenesis. Vol. I. Boca Raton, FL: CRC Press, 1984; 47–79.

No author given:

International statistical classification of diseases and related health problems. 10th revision, 2nd Ed, Vol 1. Geneva: World Health Organization, 2005; 550–564.

5.5. Tables, Figures and Figure Legends

Tables: Tables should be numbered consecutively with Arabic numerals. Each table should include a compulsory, concise explanatory title and an explanatory legend. A table should be organized with due regard for the proportion of the printed column/page. Specifically, tables which are too wide must be avoided, as these have to be printed vertically.

Figure Legends: Include Figure Legends after the reference section of the Main Text.

Figures: Articles will not be published unless the Figures fulfill journal quality criteria in terms of scientific information, general style, legibility of text and numbers, as well as electronic format and resolution. Double documentation of data in text, tables or figures is not acceptable. Always consider whether data might be better given in the text or in a table. All graphs,

drawings, and photographs are considered Figures and should be numbered in sequence with Arabic numerals. Each figure should have a legend (number and list legends after the reference section of the main text). Figures should be planned to fit the proportions of the printed page or one column’s width. Authors are encouraged to arrange micrographs into multipane

6. Early View

The European Journal of Oral Sciences is covered by Wiley-Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Articles are therefore available as soon as they are ready, rather than having to wait for the next scheduled print issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

ANEXO B – Registro de Aprovação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, referente ao Capítulo 1.

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 74/12 Fortaleza, 16 de Março de 2012.
Protocolo COMEPE nº: 35/12
Pesquisador responsável: Sérgio Lima Santiago.
Titulo do Projeto: "Efeito do fluoreto de sódio na resistência de união de sistema adesivo autocondicionante à dentina"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a pesquisa em seres humanos, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o protocolo e o TCLE do projeto supracitado na reunião do dia 15 de Março de 2012.

Outrossim, informamos, que o pesquisador deverá se comprometer a enviar o relatório final do referido projeto.

Atenciosamente,

ANEXO C – Instruções para os autores do periódico "*Journal of Biomedical Materials Research Part B: Applied Biomaterials*", referente ao Capítulo 2.

Author Guidelines

Journal of Biomedical Materials Research Part B: Applied Biomaterials

Information for Contributors

Aims and Scope

Journal of Biomedical Materials Research Part B: Applied Biomaterials is anofficial journal of the Society for Biomaterials, the Japanese Society for Biomaterials, the Australasian Society for Biomaterials, and the KoreanSociety for Biomaterials. It is a peer-reviewed journals serving the needs ofbiomaterials professionals who devise, promote, apply, regulate produce, andmarket new biomaterials and medical devices. Papers are published on devicedevelopment, implant retrieval and analysis, manufacturing, regulation ofdevices, liability and legal issues, standards, reviews of different deviceareas, and clinical applications. Published manuscript fit into one of sixcategories: original research reports, clinical device-related articles, shortresearch and development reports, review, special report, or columns andeditorials. Manuscripts from all countries are invited but must be in English.Authors are not required to be members of a Society for Biomaterials.

Types of Articles Considered for Publication

Original Research Reports: Full-length papers consisting of complete anddetailed descriptions of a research problem, the experimental approach, thefindings, and appropriate discussion. Findings should represent significantnew additions to knowledge.

Clinical Device-Related Articles: Full-length papers addressingsuch issues as material processing, device construction, regulatorymatters,clinical trials, and device retrieval.

Reviews: Scholarly and critical topic-oriented reviews thatpresent a state-of-the-art view. While most reviews are solicited, personsinterested in contributing may contact the Editor.

Special Reports: Reports of special topic-oriented symposia,deviceretrieval protocols, or other special reports not described in the abovecategories, yet of interest to the applied biomaterials research anddevelopment community. Potential contributors should contact the Editor beforesubmitting special reports.

Columns and Editorials: While columns and guest editorials arepreponderantly solicited, persons interested in becoming columnists orcontributing editorials are encouraged to contact the Editor.

Submission of Manuscripts

Online Submission:

Journal of Biomedical Materials Research Part B: Applied Biomaterials is now receiving submitted manuscripts online at http://mc.manuscriptcentral.com/jbmr-b.

Submit all new manuscripts online. Launch your web browser and go tohttp://mc.manuscriptcentral.com/jbmr-b. Check for an existing user account. Ifyou are submitting for the first time, and you do not find an existingaccount, create a new account. Follow all instructions.

At the end of a successful submission, a confirmation screen withmanuscript number will appear and you will receive an e-mail confirming thatthe manuscript has been received by the journal. If this does nothappen,please check your submission and/or contact tech support using the GetHelp Now link in the right corner of any screen.

Upon Acceptance: Manuscript files will now automatically be sentto the publisher for production. It is imperative that files be in the correctformat to avoid a delay in the production schedule.

JBMR Part B has adopted a policy that requires authors to make a statement concerning potential conflict of interest relating to theirsubmitted articles. The Editorial Board asks authors of original reports andreviews to disclose, at the time of submission: (1) any financial oremployment arrangements they may have with a company whose product figuresprominently in the submitted manuscript or with a company making a competitiveproduct; and (2) any grants or contracts from a government agency, a nonprofitfoundation, or a company supporting the preparation of the manuscript or thedescribed research. This information will be available to the reviewers of themanuscript. If the article is accepted for publication, the editor willdiscuss with the authors the manner in which such information may becommunicated to the reader.

At the time of submission, JBMR Part B asks authors to certify that all animals utilized in their research were cared for according to thepolicies and principles established by the Animal Welfare Act and the NIHGuide for Care and Use of Laboratory Animals.

Review Process: All original reports and reviews receive criticalreview by at least two reviewers with expertise in the major subject area ofthe paper. Reviewers may recommend "Acceptance as is," "Acceptance withmodification," or "Rejection." If modification is required, the manuscript isreturned to the author(s). The revised manuscript is then re-reviewed by theoriginal reviewers, and even re-revised if necessary. Differences in opinionare resolved by submission either to a third reviewer or the Editor.

Copyright/Licensing

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

For authors signing the copyright transfer agreement: If the OnlineOpen option is not selected the corresponding author will be presented with the copyright transfer agreement (CTA) to sign. The terms and conditions of the CTA can be previewed in the samples associated with the Copyright FAQs below:

CTA Terms and Conditions http://authorservices.wiley.com/bauthor/faqs_copyright.asp

For authors choosing OnlineOpen: If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):

Creative Commons Attribution Non-Commercial License OAA

Creative Commons Attribution Non-Commercial -NoDerivs License OAA

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services http://authorservices.wiley.com/bauthor/faqs_copyright.asp and visit http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html.

If you select the OnlineOpen option and your research is funded by The Wellcome Trust and members of the Research Councils UK (RCUK) you will be given the opportunity to publish

your article under a CC-BY license supporting you in complying with Wellcome Trust and Research Councils UK requirements. For more information on this policy and the Journal's compliant self-archiving policy please visit: http://www.wiley.com/go/funderstatement.
For RCUK and Wellcome Trust authors click on the link below to preview the terms and conditions of this license:
Creative Commons Attribution License OAA
To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services
http://authorservices.wiley.com/bauthor/faqs_copyright.asp and visit http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html.

Organization and File Formats

Manuscript: For optimal production, prepare manuscript text in size 12 font on 8-1/2 x 11 inch page, double-spaced, with at least 1-inch margins on all sides. Text files should be formatted as .doc or .rtf files. The results and discussion sections must be written separately and cannot be combined. Refrain from complex formatting; the Publisher will style your manuscript according tot the Journal design specifications. Do notuse desktop publishing software such as PageMaker orQuark Xpress or other software such as Latex. If you prepared your manuscript with one of these programs, export the text to a word processing format. Please make sure your word processing programs "fast save" feature is turned off. Please do not deliver files that contain hidden text: for example, do not use your word processor's automated features to create footnotes or reference lists. Manuscripts including references (but not figures or tables) should be no longer than 18 pages.

Please be sure to submit your illustrations and tables as separate files;the system will automatically create a pdf file of your paper for thereviewers.

Original research and short reports should appear in the followingorder:title page (including authors and affiliations), abstract,keywords,introduction, materials and methods, results, discussion,acknowledgments,references, figure legends. Number pages consecutively starting with the titlepage as page 1. Abbreviations must conform to those listed in Council of Biology Editors' CBE Style Manual, 5th Edition.

When mentioning a material, chemical reagent, instrument, orother product, use the generic name only. If further identification (proprietaryname, manufacturer's name and address) is absolutely required,list it in parentheses.

Title Page: List the full title of the paper and each author's full name (first name, middle initial(s), surname),department,institution, city, and state (and country if other than the UnitedStates).Indicate the name and address of the author to whom reprint requests should besent.

Abstract and Keywords: Include an abstract of about200words maximum summarizing the aims, findings, and conclusions of thepaper. Below the abstract, list five keywords or phrases that best characterize the subject matter of the manuscript.

Running Heads: Supply a short title of no more than 65 characters, including spaces and punctuations, to be used for running head copy.

References: Number references consecutively as they appear in the text.Materialaccepted for publication but not yet published may be listed intheReferences, but unpublished observations, personal communications,andmaterial submitted for publication but not yet accepted should becitedparenthetically within the text (and not included among thenumberedreferences). Style references entries using the Council of Biology Editors Style Manual, 5th Edition formats:

For journal articles:

Alexander A, Green WS. Total hip replacements: A second look. JSocBiomater 1989;45:345–366.

For books/chapters:

Ricci JL, Guichet J-M. Total hip replacement: A third look.CindraAB, Franklin DE, editors. State of the art orthopaedics, vol 3, Hips.NewYork: Wiley; 1988:56–59.

For abstracts:

Davidson GRH. Total hip replacement: A fifth look. TransABCS1987;22-341–345.

For presentations:

Goodenough T. Total hip replacement: A sixth look. Presented atthe3rd Annu Mtg Orthop Res Soc, Boston, December 5–7, 1989.

Figure Legends: Please supply complete captions for allfigures.Captions are to appear on a separate page at the end of the manuscript.

Tables: Please save Tables separately and supply numbers andtitlesfor all. All table columns should have an explanatory heading. Tablesshould besubmitted as doc or rtf files (it is preferred that tables areprepared usingWord's table edit tool.)

Illustrations: When preparing digital art, please consider:

Resolution:

The minimum requirements for resolution are:

1200 DPI/PPI for black and white images, such as line drawings or graphs.

300 DPI/PPI for picture-only photographs

600 DPI/PPI for photographs containing pictures and line elements,i.e.,text labels, thin lines, arrows.

These resolutions refer to the output size of the file; if youanticipatethat your images will be enlarged or reduced, resolutions should beadjustedaccordingly.

Formats:

For the editorial review process, GIF and JPEG filesareacceptable; upon submission of a revision, TIFF or EPS files will berequired.For the editorial review process, color images may be submitted inRGB color;upon revision, CMYK color will be required. Delivery ofproduction-qualityfiles early in the review process may facilitate smooth andrapid publicationonce a manuscript has been accepted.

Note that these file formats are not acceptable for printing: JPG,GIF,ONG, PCX, PNG, XBM, Word, and Excel. We recommend creating your graphicsinPhotoshop, Illustrator, or Freehand and importing them into yourpageapplications as TIFFs with all fonts included. Do not scan figures asJPEGsand convert to TIFFs. For further guidance on preparing digital figurefiles,authors are encouraged to visit http://cjs.cadmus.com/da/applications.asp .

To ensure that your digital graphics are suitable for printpurposes,please go to RapidInspector™ at http://rapidinspector.cadmus.com/zwi/index.jsp. Thisfree,stand-alone software application will help you to inspect andverifyillustrations right on your computer.

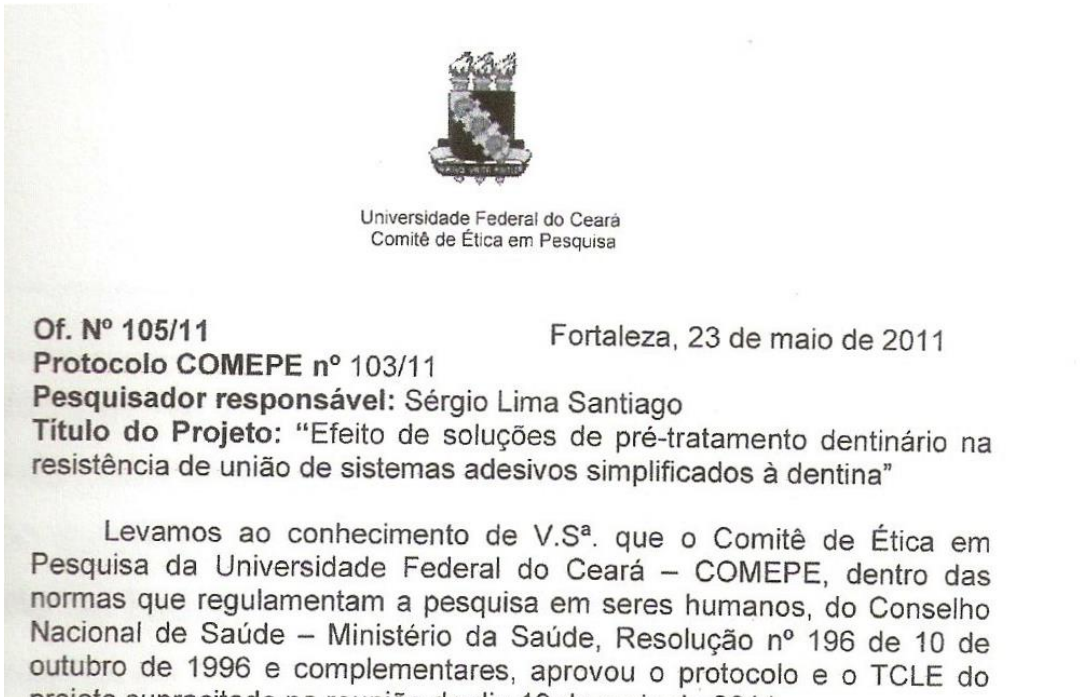
A legend must be provided for each illustration and must defineallabbreviations used therein. Legends should be placed at the end ofthemanuscript text file.

Color Illustrations: Color figures are generally printedinthe Journal at the author's expense. The published will provide costestimatesprior to printing. A limited number of color figures that are ofcriticalimportance and that significantly enhance the presentation will beconsideredfor publication at the publisher's expense subject toeditorialrecommendation. Final decision on publication of color figures willbe at thediscretion of the Editor. All color figures will be reproduced infull colorin the online edition of the journal at no cost to authors. Forbestreproduction, bright, clear colors should be used. Dark colors against a dark background do not reproduce well; please place your color images againstawhite background wherever possible.

Reprints: Reprints may be ordered at https://caesar.sheridan.com/reprints/redir.php?pub=10089&acro=JEMB.

Note to NIH Grantees: Pursuant to NIH mandate, Wiley-Blackwell will post the accepted version of contributions authored by NIH grant-holders to PubMed Central upon acceptance. This accepted version will be made publicly available 12 months after publication. For further information, see www.wiley.com/go/nihmandate.

ANEXO D – Registro de Aprovação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, referente ao Capítulo 2.



Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 105/11 Fortaleza, 23 de maio de 2011
Protocolo COMEPE nº 103/11
Pesquisador responsável: Sérgio Lima Santiago
Título do Projeto: "Efeito de soluções de pré-tratamento dentinário na resistência de união de sistemas adesivos simplificados à dentina"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a pesquisa em seres humanos, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o protocolo e o TCLE do

ANEXO E – Instruções para os autores do periódico "*Brazilian Dental Journal*", referente ao Capítulo 3.

Author Guidelines

Brazilian Dental Journal

Information for Contributors

Scope and policy

The Brazilian Dental Journal publishes Full-Length Papers, Short Communications and Case Reports, dealing with dentistry or related disciplines. Only original papers will be considered for publication. In submitting a manuscript, the authors should state in the cover letter that the

material has not been published previously and is not under consideration by another journal in either electronic or printed versions.

ELECTRONIC ADDRESS FOR SUBMISSION
http://mc04.manuscriptcentral.com/bdj-scielo

MANUSCRIPTS MUST BE SUBMITTED IN ENGLISH. Authors whose primary language is not English must have their manuscript reviewed by someone proficient in English. Manuscripts accepted for publication will be submitted to the Technical Review for revision of English grammar and scientific writing and to fit the text into the Journal's standards. The cost of the Technical Review will be charged to the authors. Submission of a manuscript to BDJ implies the acceptance of these terms. The decision of acceptance for publication relies on the Editors and is based on the recommendation of the Editorial Board and/or ad hoc reviewers. Authors of manuscripts not recommended for publication will receive an email explaining the decision. The concepts emitted in the papers published in the BDJ are the sole responsibility of the authors, not necessarily reflecting the Editorial Board's opinion.

Form and preparation of manuscripts
THE FOLLOWING GUIDELINES MUST BE FOLLOWED CAREFULLY.

General

- The authors must submit the manuscript in Word and in PDF, comprising the title page, text, tables, figure captions and figures (photographs, micrographs, radiographs, schematic drawings, graphs, computer-generated images, etc).
- The manuscript must be typed in Times New Roman 12 font, with 1.5 spacing, 2.5-cm margins at each side. DO NOT USE bold letters, watermarks or other resources to make the text visually attractive.

Pages should be numbered consecutively, starting with the summary.
Full-length manuscripts are assembled in the following sections:
1) Title Page
2) Summary and Key Words
3) Introduction; Material and Methods; Results; Discussion
4) Summary in Portuguese (an item necessary for Latin American Indexing Services that will be provided for non-Brazilian authors by the Journal)

5) Acknowledgements (if any)
6) References
7) Tables
8) Figure captions
9) Figures

All titles of sections (Introduction, Material and Methods, etc) must be capitalized in regular font type (not bold).
Results and Discussion MUST NOT be joined in a single section.
Short Communications and Case Reports should be divided into appropriate sections.
Products, equipments and materials: the trade name must be followed by the manufacturer's name, city, state and country, within parentheses upon first mention. For further mentions, only the manufacturer's name is required.
All abbreviations must be explained at first mention.

Title page

The first page must contain the title of the manuscript, a short title (maximum of 40 characters, to be used as a running head), author(s) name(s) (no more than 6) and their Department(s), School(s) and/or University (s). DO NOT INCLUDE the author's titles (DDS, MSc, PhD, etc.) or position (Professor, Graduate student, etc.).
Provide the name and complete address of the corresponding author (inform email, telephone and fax numbers).
The title page must be uploaded at the website as a separate file (not included in the body of the manuscript).

Manuscript

The first page of the manuscript must contain: title of the manuscript, short tile with no more than 40 characters, and NO authors' names or identification.

Summary

The second page should contain a summary of no more than 250 words, stating the aims, methods, results, and any conclusions drawn from the study. Do not use topics and paragraphs and do not cite references in the Summary.

A list of key words (no more than 5) should be included below the summary in lowercase letters, separated by commas.

Introduction

Summarize the purpose of the study, giving only pertinent references. Do not review existing literature extensively. State clearly the working hypothesis.

Material and Methods

Material and methods should be presented in sufficient detail to allow confirmation of the observations. Indicate the statistical methods used, if applicable.

Results

Present the results in a logical sequence in the text, tables and figures, emphasizing the important information.

Do not repeat in the text data contained in the tables and illustrations. The important observations should be emphasized.

Do not repeat the same data in tables and figures.

Describe the statistical data in this section.

Discussion

Summarize the findings without repeating in detail the data given in the Results section.

Relate your observations to other relevant studies and point out the implications of the findings and their limitations. Cite pertinent studies.

Present your conclusions at the end of the Discussion, indicating how your study is pertinent and/or its clinical implications. Presentation of the conclusions in topics should be avoided.

Summary in Portuguese (for Brazilian authors only)

The Summary in Portuguese should be IDENTICAL to the English version (Summary). DO NOT INCLUDE title and key words in Portuguese.

Acknowledgements

Financial support by government agencies should be acknowledged. If appropriate, technical assistance or assistance from colleagues may be acknowledged.

References

References must follow the Journal's style. Authors should refer to a current issue of the BDJ for guidance on reference citation and presentation of the reference list.

References must be numbered consecutively in the text in order of citation, within parentheses, without space between numbers: (1), (3,5,8), (10-15). DO NOT USE superscript numbers.

For papers with two authors, cite both authors in the text, as follows: Ex: "According to Santos and Silva (1)...". If there are more than 3 authors, cite only the first author and add "et al.". Ex: "Pécora et al. (2) reported that..."

All authors of each paper should be included in the Reference List unless there are 7 or more. In this case, the first 6 authors should be given, followed by "et al.".

The reference list must be typed at the end of the manuscript in numerical sequence. No more than 25 references may be cited.

Citation of abstracts and books, as well as articles published in non-indexed journals should be avoided, unless absolutely necessary. Do not cite references in Portuguese.

Abbreviations of journal titles should conform to those used in Dental Index. The style and punctuation of references must follow the format illustrated below:

Journal articles

1. Lea SC, Landini G, Walmsley AD. A novel method for the evaluation of powered toothbrush oscillation characteristics. Am J Dent 2004;17:307-309.

Book

2. Shafer WG, Hine MK, Levy BM. A Textbook of Oral Pathology. 4th ed. Philadelphia: WB Saunders; 1983.

Chapter in a Book

3. Walton RE, Rotstein I. Bleaching discolored teeth: internal and external. In: Principles and Practice of Endodontics. Walton RE (Editor). 2nd ed. Philadelphia: WB Saunders; 1996. p 385-400.

Tables

Each table with its title must be typed after the text. Tables should be numbered with Arabic numerals. DO NOT USE vertical lines, bold letters and capital letters (except the initials).

The corresponding title should appear at the top of each table.

Tables must contain all necessary information and be understandable without allusions to the text.

Figures

BDJ WILL NOT ACCEPT FIGURES EMBEDDED IN FILES ORIGINATED IN TEXT-EDITING SOFTWARE (WORD OR SIMILAR) OR FIGURES ORIGINATED IN POWER POINT.

The digital files of the images should be generated in Photoshop, Corel or any other image-editing software and saved in the CD-ROM. Image files should have TIFF extension and 300 dpi minimum resolution. Only BLACK & WHITE figures are accepted. Save the figures in the CD-ROM.

Lettering and identifying marks must be clear and sharp, and the critical areas of x-rays and photomicrographs must be demarcated and/or isolated.

Separate parts of composite figures must be labeled with capital letters (A, B, C, etc). Single figures and composite figures must have minimum width of 8 cm and 16 cm, respectively.

Figure captions should be numbered with Arabic numerals and typed on a separate page, after the lists of references or after the tables (if any).

ANEXO F – Instruções para os autores do periódico "*International Journal of Pharmaceutics*", referente ao Capítulo 4.

International Journal of Pharmaceutics

Information for Contributors

The International Journal of Pharmaceutics publishes innovative papers, reviews, mini-reviews, rapid communications and notes dealing with physical, chemical, biological, microbiological and engineering studies related to the conception, design, production, characterisation and evaluation of drug delivery systems in vitro and in vivo. "Drug" is defined as any therapeutic or diagnostic entity, including oligonucleotides, gene constructs and radiopharmaceuticals.

Areas of particular interest include: pharmaceutical nanotechnology; physical pharmacy; polymer chemistry and physical chemistry as applied to pharmaceutics; excipient function and characterisation; biopharmaceutics; absorption mechanisms; membrane function and transport; novel routes and modes of delivery; responsive delivery systems, feedback and control mechanisms including biosensors; applications of cell and molecular biology to drug delivery;

prodrug design; bioadhesion (carrier-ligand interactions); and biotechnology (protein and peptide formulation and delivery).

Note: For details on pharmaceutical nanotechnology, see Editorials in 279/1-2 281/1, and 288/1.

Types of paper

(1) Full Length Manuscripts

(2) Rapid Communications

(a) These articles should not exceed 1500 words or equivalent space.
(b) Figures should not be included otherwise delay in publication will be incurred.
(c) Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.

(3) Notes

Should be prepared as described for full length manuscripts, except for the following:

(a) The maximum length should be 1500 words, including figures and tables.
(b) Do not subdivide the text into sections. An Abstract and reference list should be included.

(4) Reviews and Mini-Reviews

Suggestions for review articles will be considered by the Review-Editor. "Mini-reviews" of a topic are especially welcome.

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Human and animal rights

If the work involves the use of animal or human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans http://www.wma.net/en/30publications/10policies/b3/index.html; EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923.

Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/sharingolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including

electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Contributors

Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:
Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Article transfer service

This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information about this can be found here: http://www.elsevier.com/authors/article-transfer-service.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted third party reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some authors may also be reimbursed for associated publication fees. To learn more about existing agreements please visit http://www.elsevier.com/fundingbodies.

Open access

This journal offers authors a choice in publishing their research:

Open access
• Articles are freely available to both subscribers and the wider public with permitted reuse
• An open access publication fee is payable by authors or on their behalf e.g. by their research funder or institution
Subscription
• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs (http://www.elsevier.com/access).
• No open access publication fee payable by authors.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)
Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)
For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The open access publication fee for this journal is USD 3000, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Authors must state in a covering letter when submitting papers for publication the novelty embodied in their work or in the approach taken in their research. Routine bioequivalence studies are unlikely to find favour. No paper will be published which does not disclose fully the nature of the formulation used or details of materials which are key to the performance of a product, drug or excipient. Work which is predictable in outcome, for example the inclusion of another drug in a cyclodextrin to yield enhanced dissolution, will not be published unless it provides new insight into fundamental principles.

Note:
The choice of general classifications such as "drug delivery" or "formulation" are rarely helpful when not used together with a more specific classification.

Referees

Please submit, with the manuscript, the names, addresses and e-mail addresses of at least four potential reviewers. Good suggestions lead to faster processing of your paper. Please note:
Reviewers who do not have an institutional e-mail address will only be considered if their affiliations are given and can be verified.
Please ensure that the e-mail addresses are current.
International reviewers who have recently published in the appropriate field should be nominated, and their areas of expertise must be stated clearly.
Note that the editor retains the sole right to decide whether or not the suggested reviewers are contacted.
To aid the editorial process when suggested reviewers are not chosen or decline to review, ensure that the classifications chosen as the field of your paper are as detailed as possible. It is not sufficient to state "drug delivery" or "nanotechnology" etc.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.
• Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

The abstract must not exceed 200 words.

Graphical abstract

A Graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more, but should be readable on screen at a size of 200 × 500 pixels (at 96 dpi this corresponds to 5 × 13 cm). Bear in mind readability after reduction, especially if using one of the figures from the article itself. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Chemical compounds

You can enrich your article by providing a list of chemical compounds studied in the article. The list of compounds will be used to extract relevant information from the NCBI PubChem Compound database and display it next to the online version of the article on ScienceDirect. You can include up to 10 names of chemical compounds in the article. For each compound, please provide the PubChem CID of the most relevant record as in the following example: Glutamic acid (PubChem CID:611). The PubChem CIDs can be found via http://www.ncbi.nlm.nih.gov/pccompound. Please position the list of compounds immediately below the 'Keywords' section. It is strongly recommended to follow the exact text formatting as in the example below:
Chemical compounds studied in this article
Ethylene glycol (PubChem CID: 174); Plitidepsin (PubChem CID: 44152164); Benzalkonium chloride (PubChem CID: 15865)
More information is available at: http://www.elsevier.com/PubChem.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Database linking

Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you

submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications that can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions
Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press'

implies that the item has been accepted for publication and a copy of the title page of the relevant article must be submitted.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference

style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: All citations in the text should refer to:

1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;
2. Two authors: both authors' names and the year of publication;
3. Three or more authors: first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In

order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

It is hoped that this list will be useful during the final checking of an article prior to sending it to the journal's Editor for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One Author designated as corresponding Author:

• E-mail address

• Full postal address

• Telephone and fax numbers

All necessary files have been uploaded

• Keywords

• All figure captions

• All tables (including title, description, footnotes)

Further considerations:

• Use continuous line numbering (every 5 lines) to facilitate reviewing of the manuscript.

• Manuscript has been "spellchecked" and "grammar-checked"

• References are in the correct format for this journal

• All references mentioned in the Reference list are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Web)

• Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print or to be reproduced in color on the Web (free of charge) and in black-and-white in print

• If only color on the Web is required, black and white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

ANEXO G – Instruções para os autores do periódico "*Journal of Dentistry*", referente ao Capítulo 5.

ELSEVIER

JOURNAL OF DENTISTRY

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0300-5712

DESCRIPTION

The Journal of Dentistry is the leading international dental journal within the field of **Restorative Dentistry**. Placing an emphasis on publishing novel and high-quality research papers, the Journal aims to influence the practice of **dentistry** at clinician, research, industry and policy-maker level on an international basis.

Topics covered include the management of **dental disease**, **periodontology**, **endodontology**, **operative dentistry**, fixed and removable **prosthodontics**, **dental biomaterials** science, long-term clinical trials including **epidemiology** and **oral health**, technology transfer of new scientific instrumentation or procedures, as well as clinically relevant **oral biology** and translational research.

The Journal of Dentistry will publish original scientific research papers including short communications. It is also interested in publishing review articles and leaders in themed areas which will be linked to new scientific research. Conference proceedings are also welcome and expressions of interest should be communicated to the Editor.

AUDIENCE

Those interested in developments in oral and dental research including practising clinicians, dental researchers, clinical academics, those involved in dental industry, and policy-makers relevant to the practice of dentistry.

IMPACT FACTOR

2013: 2.840 © Thomson Reuters Journal Citation Reports 2014

ABSTRACTING AND INDEXING

Abstracts on Hygiene and Communicable Diseases
Agris
BIOSIS
Cancerlit
Chemical Abstracts
Current Contents
Current Contents/Clinical Medicine
Current Titles in Dentistry
Dairy Science Abstracts
Index Dental Literature
MEDLINE®
Index Veterinarius
Medical Documentation Service
Dental Abstracts
Environmental Studies
Nutrition Research Newsletter
Pascal
Research Alert
Review of Medical and Veterinary Mycology
SCISEARCH
Science Citation Index
Social SciSearch
Tropical Diseases Bulletin
UnCover
Veterinary Bulletin
CABI Information
TOXFILE
BIOSIS Previews
Inpharma Weekly
PharmacoEconomics and Outcomes News
Reactions Weekly
Review of Aromatic and Medicinal Plants
Scopus
Global Health
Nutrition Abstracts and Reviews Series
Pharm-line
ISI Science Citation Index
Chemical Industry Notes
Adis Clinical Trials Insight
CSA Life Sciences Abstracts
Dialog Journal Name Finder
ONTAP MEDLINE

EDITORIAL BOARD

GUIDE FOR AUTHORS

INTRODUCTION

Editor-in-Chief
Christopher D. Lynch
School of Dentistry
Cardiff University
Heath Park, Cardiff,
CF14 4NQ, UK
Email: lynchcd@cardiff.ac.uk

Editorial Office
Elsevier Ltd
Stover Court
Bampfylde Street
Exeter
EX1 2AH, UK
Tel: +44 (0) 1392 285879
Fax: +44 (0) 1865 853132
E-mail: JOD@elsevier.com

The Journal of Dentistry is the leading international dental journal within the field of Restorative Dentistry. Placing an emphasis on publishing novel and high-quality research papers, the Journal aims to influence the practice of dentistry at clinician, research, industry and policy-maker level on an international basis.

Topics covered include the management of dental disease, periodontology, endodontology, operative dentistry, fixed and removable prosthodontics, and dental biomaterials science, long-term clinical trials including epidemiology and oral health, dental education, technology transfer of new scientific instrumentation or procedures, as well clinically relevant oral biology and translational research. Submissions are welcomed from other clinically relevant areas, however, the Journal places an emphasis on publishing high-quality and novel research.

Queries in relation to manuscript content should be directed to the Journal Editorial Office in the first instance.

Submissions

Authors are requested to submit their original manuscript and figures via the online submission and editorial system for Journal of Dentistry. Using this online system, authors may submit manuscripts and track their progress through the system to publication. Reviewers can download manuscripts and submit their opinions to the editor. Editors can manage the whole submission/review/revise/publish process. Please register at: http://ees.elsevier.com/jjod

Types of paper

Contributions falling into the following categories will be considered for publication:- Original Research Reports: maximum length 6 printed pages approximately 20 typescript pages, including illustrations and tables.
- Review articles: maximum length 10 printed pages, approximately 33 typescript pages, including illustrations and tables.
- Short communication for rapid publication: maximum length 2 printed pages, approximately 7 typescript pages, including illustrations.
- Letters providing informed comment and constructive criticism of material previously published in the Journal.

All typescripts must be accompanied by a Permission Note. This is a letter signed by each author (not just the corresponding author), affirming that the paper has been submitted solely to Journal of Dentistry and that it is not concurrently under consideration for publication in another journal. Prospective authors should confirm that the submitted work, including images, are original. Authors are reminded that if included images (e.g. Tables and Figures) have been previously published may require copyright permission.

Authorship

Only those persons who have made a significant contribution to the manuscript submitted should be listed as authors. The Editor-in-Chief expects that a manuscript should normally have no more than 6 authors, unless a case is made by the corresponding author within the article cover letter to include other authors. All of the named authors should have been involved in the work leading to the publication of the paper and should have read the paper before it is submitted for publication.

BEFORE YOU BEGIN

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Human and animal rights

If the work involves the use of animal or human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans http://www.wma.net/en/30publications/10policies/b3/index.html; EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Conflict of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there are no conflicts of interest then please state this: 'Conflicts of interest: none'. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Clinical trial results

In line with the position of the International Committee of Medical Journal Editors, the journal will not consider results posted in the same clinical trials registry in which primary registration resides to be prior publication if the results posted are presented in the form of a brief structured (less than 500

words) abstract or table. However, divulging results in other circumstances (e.g., investors' meetings) is discouraged and may jeopardise consideration of the manuscript. Authors should fully disclose all posting in registries of results of the same or closely related work.

Reporting clinical trials

Randomized controlled trials should be presented according to the CONSORT guidelines. At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization, withdrawal and completion, and a detailed description of the randomization procedure. The CONSORT checklist and template flow diagram can be found on http://www.consort-statement.org.

Registration of clinical trials

Registration in a public trials registry is a condition for publication of clinical trials in this journal in accordance with International Committee of Medical Journal Editors (ICMJE, http://www.icmje.org) recommendations. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration.

Article transfer service

This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information about this can be found here: http://www.elsevier.com/authors/article-transfer-service.

Copyright

This journal offers authors a choice in publishing their research: Open access and Subscription.

For subscription articles

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.
Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For open access articles

Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Retained author rights

As an author you (or your employer or institution) retain certain rights. For more information on author rights for:
Subscription articles please see http://www.elsevier.com/journal-authors/author-rights-and-responsibilities.
Open access articles please see http://www.elsevier.com/OAauthoragreement.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

Open access

This journal offers authors a choice in publishing their research:

Open access
- Articles are freely available to both subscribers and the wider public with permitted reuse
- An open access publication fee is payable by authors or their research funder

Subscription
- Articles are made available to subscribers as well as developing countries and patient groups through our access programs (http://www.elsevier.com/access)
- No open access publication fee

All articles published open access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by your choice of one of the following Creative Commons user licenses:

Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA): for non-commercial purposes, lets others distribute and copy the article, to create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, do not modify the article in such a way as to damage the author's honor or reputation, and license their new adaptations or creations under identical terms (CC BY-NC-SA).

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND): for non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

Elsevier has established agreements with funding bodies, http://www.elsevier.com/fundingbodies. This ensures authors can comply with funding body open access requirements, including specific user licenses, such as CC BY. Some authors may also be reimbursed for associated publication fees. If you need to comply with your funding body policy, you can apply for the CC BY license after your manuscript is accepted for publication.

To provide open access, this journal has a publication fee which needs to be met by the authors or their research funders for each article published open access.
Your publication choice will have no effect on the peer review process or acceptance of submitted articles.

The open access publication fee for this journal is **$1700**, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article
Please submit your article via .

Referees

Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

Double-blind review

This journal uses double-blind review, which means that both the reviewer and author name(s) are not allowed to be revealed to one another for a manuscript under review. The identities of the authors are concealed from the reviewers, and vice versa. For more information please refer to http://www.elsevier.com/reviewers/peer-review. To facilitate this, please include the following separately:
Title page (with author details): This should include the title, authors' names and affiliations, and a complete address for the corresponding author including telephone and e-mail address.
Blinded manuscript (no author details): The main body of the paper (including the references, figures, tables and any Acknowledgements) should not include any identifying information, such as the authors' names or affiliations.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Introduction
The introduction must be presented in a structured format, covering the following subjects, although not under subheadings: succinct statements of the issue in question, and the essence of existing knowledge and understanding pertinent to the issue. In keeping with the house style of Journal of Dentistry, the final paragraph of the introduction should clearly state the aims and/or objective of the work being reported. Prospective authors may find the following form of words to be helpful: "The aim of this paper is to ..." Where appropriate, a hypothesis (e.g. null or a priori) should then be stated.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

The **title page** should contain the following information:
- Title of paper
- Short title
- Name(s), job titles and address(es) of author(s) (no academic degrees necessary)
- Name, address, telephone, fax and e-mail of the corresponding author
- Up to 6 keywords

Spelling: International English.

Authors are urged to write as concisely as possible.

The house style of Journal of Dentistry requires that articles should be arranged in the following order: Title, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements, References, Tables, Figures. A **cover letter** should accompany the new manuscript submission, within which the authors should indicate the significance of the work being submitted in a statement no more than 100 words. A signed **permission note** (details below) must also be included.

Abstract: should not exceed 250 words and should be presented under the following subheadings: Objectives, Methods; Results; Conclusions (For Reviews: Objectives; Data; Sources; Study selection; Conclusions). A 50 word 'Clinical Significance' statement should appear at the end of the abstract advising readers of the clinical importance and relevance of their work. These subheadings should appear in the text of the abstract. Please repeat the title of the article at the top of the abstract page.

Introduction: must be presented in a structured format, covering the following subjects, although not under subheadings: succinct statements of the issue in question, and the essence of existing knowledge and understanding pertinent to the issue. In keeping with the house style of Journal of Dentistry, the final paragraph of the introduction should clearly state the aims and/or objective of the work being reported. Prospective authors may find the following form of words to be helpful: "The aim of this paper is to ..." Where appropriate, a hypothesis (e.g. null or a priori) should then be stated.

Keywords: up to 6 keywords should be supplied.

Abbreviations and acronyms: terms and names to be referred to in the form of abbreviations or acronyms must be given in full when first mentioned.

Units: SI units should be used throughout. If non-SI units must be quoted, the SI equivalent must immediately follow in parentheses.

The complete names of individual teeth must be given in the test. In tables and legends for illustrations individual teeth should be identified using the FDI two-digit system.

Statistics
Statistical methods should be described with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, findings should be quantified and appropriate measures of error or uncertainty (such as confidence intervals) given. Details about eligibility criteria for subjects, randomization and the number of observations should be included. The computer software and the statistical method(s) used should be specified with references to standard works when possible (with pages specified). See http://www.icmje.org/manuscript_1prepare.html for more detailed guidelines.

References: These should appear in the text in numerical order and should follow a modified form of the Vancouver Reference system (details may be found at http://www.icmje.org/index.html#reference. Please note that the house style of the Journal of Dentistry is different from the standard Vancouver reference style in that it includes a requirement:

- to refer to the name of the Journal in full
- to put the name of the Journal in Italics
- to put the volume number in bold

Examples as follows:

Journal articles

Lynch CD, Frazier KB, McConnell RJ, Blum IR, Wilson NHF. State-of-the-art techniques in Operative Dentistry: contemporary teaching of posterior composites in UK and Irish dental schools. British Dental Journal 2010; **209**: 129 - 36.

Wilson NHF, Mjör I. The teaching of class I and class II direct composite restorations in European dental schools. Journal of Dentistry 2000; **28**: 15-21.

Please note that in-press/ accepted articles that are awaiting assignment of page numbers should be cited including their DOI number (Digital Object Identifier), for example:

Books

Lynch CD. Successful posterior composites. London: Quintessence Publishing Co., 2008.

Book chapters

Phillips SJ, Whisnant JP. The role of dentine under restorations. In: Laragh JH, Brenner BM, editors. The science of restorative dentistry. 2nd ed. Oxford: Elsevier; 2003. p.266-78.

If there are seven or more authors please list the first six and et al., otherwise list all authors. Journal titles should be given in full. If websites are used as references, the full URL should be cited, along with the date on which it was accessed.

Illustrations: should be submitted electronically using appropriate commercial software. Prospective authors should follow the relevant guidelines (available from: http://www.elsevier.com/artworkinstructions). In addition, it is noted that while authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, journals published by Elsevier apply the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Abstract

The Abstract should not exceed 250 words and should be presented under the following subheadings: Objectives, Methods; Results; Conclusions (For Reviews: Objectives; Data; Sources; Study selection; Conclusions). A 50 word 'Clinical Significance' statement should appear at the end of the abstract advising readers of the clinical importance and relevance of their work. These subheadings should appear in the text of the abstract. Please repeat the title of the article at the top of the abstract page.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images and in accordance with all technical requirements: Illustration Service.

Keywords

Provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Artwork

Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the printed version.
• Submit each illustration as a separate file.

A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications that can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Illustration services

Elsevier's WebShop (http://webshop.elsevier.com/illustrationservices) offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style

References should appear in the text in numerical order and should follow a modified form of the Vancouver Reference system (details may be found at http://www.icmje.org/index.html#reference. Please note that the house style of the Journal of Dentistry is different from the standard Vancouver reference style in that it includes a requirement:

- to refer to the name of the Journal in full
- to put the name of the Journal in Italics
- to put the volume number in bold

Examples as follows:

Journal articles

Lynch CD, Frazier KB, McConnell RJ, Blum IR, Wilson NHF. State-of-the-art techniques in Operative Dentistry: contemporary teaching of posterior composites in UK and Irish dental schools. British Dental Journal 2010; **209**: 129 - 36.

Wilson NHF, Mjör I. The teaching of class I and class II direct composite restorations in European dental schools. Journal of Dentistry 2000; **28**: 15-21.

Please note that in-press/ accepted articles that are awaiting assignment of page numbers should be cited including their DOI number (Digital Object Identifier), for example:

Books

Lynch CD. Successful posterior composites. London: Quintessence Publishing Co., 2008.

Book chapters

Phillips SJ, Whisnant JP. The role of dentine under restorations. In: Laragh JH, Brenner BM, editors. The science of restorative dentistry. 2nd ed. Oxford: Elsevier; 2003. p.266-78.

If there are seven or more authors please list the first six and et al., otherwise list all authors. Journal titles should be given in full. If websites are used as references, the full URL should be cited, along with the date on which it was accessed.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Video data
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary data
Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist
The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.
Ensure that the following items are present:
One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

• Phone numbers
All necessary files have been uploaded, and contain:
• Keywords
• All figure captions
• All tables (including title, description, footnotes)
Further considerations
• Manuscript has been 'spell-checked' and 'grammar-checked'
• References are in the correct format for this journal
• All references mentioned in the Reference list are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Web)
• Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
• If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes
For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):
http://dx.doi.org/10.1016/j.physletb.2010.09.059
When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor.It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author, at no cost, will be provided with a personalized link providing 50 days free access to the final published version of the article on ScienceDirect. This link can also be used for sharing via email and social networks. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com/myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/booklets).

The decision of the Editor-in-Chief is final in relation to all manuscript submissions.

AUTHOR INQUIRIES

You can track your submitted article at http://help.elsevier.com/app/answers/detail/a_id/89/p/8045/. You can track your accepted article at http://www.elsevier.com/trackarticle. You are also welcome to contact Customer Support via http://support.elsevier.com.

ANEXO H – Registro de Aprovação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, referente ao Capítulo 5.

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA INCORPORAÇÃO DE CATEQUINA EM SISTEMA ADESIVO CONVENCIONAL NA RESISTÊNCIA DE UNIÃO À DENTINA

Pesquisador: Nadine Luisa Soares de Lima Guimarães

Área Temática:

Versão: 1

CAAE: 22468813.0.0000.5054

Instituição Proponente: Departamento de Odontologia Restauradora

Patrocinador Principal: Departamento de Odontologia Restauradora

DADOS DO PARECER

Número do Parecer: 459.659

Data da Relatoria: 14/11/2013

Apresentação do Projeto:

Os materiais adesivos são bastante utilizados na odontologia e têm demonstrado excelentes resultados na prática clínica diária. Atualmente, os sistemas adesivos são classificados em convencionais e autocondicionantes.O presente estudo como objetivo comparar o efeito do flavonóide Epigalocatequina-3-galato (EGCG) incorporado ao sistema adesivo convencional ou como pré-tratamento da dentina na resistência de união, através de ensaios de microtração imediatos e após armazenagem por 6 e 12 meses.

Serão utilizados 40 terceiros molares humanos hígidos. Após remoção do esmalte e dentina oclusal, utilização da lixa de carbeto de silício (SiC) Nº 100 acoplada em uma politriz elétrica para completa remoção de esmalte e a de Nº 600 durante 1 minuto para gerar um padrão de smear layer em todos os espécimes, os espécimes serão distribuídos aleatoriamente em 5 Grupos (n=8): Grupo I (Água destilada + Adper¿ Single Bond 2®); Grupo II (Água destilada + EGCG puro incorporado ao Adper¿ Single Bond 2®); Grupo III (EGCG em cápsula incorporado ao Adper¿ Single Bond 2®); Grupo IV (Solução aquosa de EGCG + Adper¿ Single Bond 2®) e; Grupo V (Solução de EGCG cápsula + Adper¿ Single Bond 2®). Primeiramente,

Endereço: Rua Cel. Nunes de Melo, 1127
Bairro: Rodolfo Teófilo **CEP:** 60.430-270
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

será feito condicionamento das superfícies dentárias com ácido fosfórico por 15 segundos, em seguida essas superfícies serão lavadas com spray
ar/água por 15 segundos, secas por 30 segundos, e reumidecidas com as soluções de limpeza de acordo com cada Grupo (15 ¿L, ativamente por
60s). O excesso será removido com papéis absorventes, deixando a superfície dentinária visivelmente úmida. Na sequência, será aplicado o sistema
adesivo Adper¿ Single Bond 2® de formulação específica para cada Grupo, de acordo com as recomendações do fabricante. A porção coronária
será reconstruída com 4 incrementos (1 mm cada) de resina composta (Filtek Z250XT®). Após estocagem em água destilada a 37 ºC por 24h,
espécimes em forma de palito (constituídos de resina e dentina unidas pela interface adesiva) serão obtidos por meio de cortes seriados dos dentes.
Após a obtenção dos palitos, estes serão submetidos a uma força de tração em uma máquina de ensaios universais à velocidade de 0,5 mm/ minuto
até que ocorra a fratura. Os palitos que irão ser testados após 6 meses e 1 ano ficarão armazenados em em água destilada. Serão avaliados os
modos de fratura com o auxílio de uma Lupa Estereoscópica e classificadas em: 1) Falha coesiva em resina composta (FCR); 2) Falha coesiva em
dentina (FCD); 3) Falha adesiva (FA); e 4) Falha mista (FM). A partir dos dados obtidos em Mpa será realizado teste de normalidade e, se
constatada a presença de distribuição normal das amostras, os dados serão submetidos ao teste ANOVA (Análise de Variância) para comparação
entre os grupos e em seguida, eventuais diferenças entre os grupos serão analisadas por teste de comparação múltipla. No caso dos resultados
falharem no teste de normalidade, será aplicado teste Kruskal-Wallis. O nível de significância será de p0,05 em todas as situações.

Objetivo da Pesquisa:
Comparar o efeito do flavonóide Epigalocatequina-3-galato (EGCG) incorporado ao sistema adesivo convencional ou como pré-tratamento da
dentina na resistência de união, através de ensaios de microtração imediatos e após armazenagem por 6 e 12 meses.

Avaliação dos Riscos e Benefícios:
Riscos:

Endereço: Rua Cel. Nunes de Melo, 1127
Bairro: Rodolfo Teófilo CEP: 60.430-270
UF: CE Município: FORTALEZA
Telefone: (85)3366-8344 Fax: (85)3223-2903 E-mail: comepe@ufc.br

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Plataforma Brasil

Continuação do Parecer: 459.659

Não há riscos envolvidos pois trata-se de uma pesquisa em laboratório com dentes humanos extraídos por motivos que não envolvem este estudo.

Benefícios:

De acordo com os resultados obtidos, será possível propor novas técnicas e materiais a serem usados na odontologia restauradora.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante na área de dentística com metodologia clara e objetiva e compatível com o objetivo do trabalho

Considerações sobre os Termos de apresentação obrigatória:

Apresentou: folha de rosto, dispensa do TCLE, termo de doação de dentes, orçamento detalhado, currículo Lattes, carta de apreciação ao COMEPE, cronograma, autorização do local onde será realizada a pesquisa, declaração de concordância, declaração de vínculo da aluna no mestrado, declaração de custeio da pesquisa, projeto com anexos.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Não se aplica.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

FORTALEZA, 18 de Novembro de 2013

Assinador por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador)

Endereço: Rua Cel. Nunes de Melo, 1127
Bairro: Rodolfo Teófilo CEP: 60.430-270
UF: CE Município: FORTALEZA
Telefone: (85)3366-8344 Fax: (85)3223-2903 E-mail: comepe@ufc.br