RENATA BORGES RODRIGUES

Complicações da radioterapia no esmalte e dentina humanos: análise da adesão, composição química e propriedades mecânicas

Complications of radiotherapy in human enamel and dentin: adhesion analysis, chemical composition and mechanical properties

Tese apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia, como requisito parcial para obtenção do título de Doutor em Odontologia na área de Clínica Odontológica Integrada.

Uberlândia, 2019

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Orientadora: Profª. Drª. Veridiana Resende Novais Simamoto

Banca Examinadora:

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Profª. Drª. Gisele Rodrigues da Silva - Faculdade de Odontologia – Universidade Federal de Uberlândia

Profª. Drª. Priscilla Barbosa Ferreira Soares - Faculdade de Odontologia – Universidade Federal de Uberlândia

Profª. Drª. Linda Wang – Faculdade de Odontologia de Bauru – Universidade de São Paulo

Profª. Drª. Fabíola Galbiatti de Carvalho Carlo – Departamento de Odontologia – Universidade Federal de Juiz de Fora

Uberlândia, 2019

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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ATA

Ata da defesa de TESE DE DOUTORADO junto ao Programa de Pós-graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Uberlândia.

Defesa de: Tese de Doutorado COPOD

Data: 22/02/2019

Discente: **Renata Borges Rodrigues (11513ODO0014)**

Título do Trabalho: Complicações da radioterapia no esmalte e dentina humanos: análise da adesão, composição química e propriedades mecânicas

Área de concentração: Clínica Odontológica Integrada.

Linha de pesquisa: Propriedades físicas e biológicas dos materiais odontológicos e das estruturas dentais

Projeto de Pesquisa de vinculação: Propriedades físicas e biológicas dos materiais odontológicos e das estruturas dentais

As **quatorze horas** do d**ia vinte e dois de fevereiro de 2019** no Anfiteatro Bloco 4L Anexo A, sala 23 Campus Umuarama da Universidade Federal de Uberlândia, reuniu-se a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em janeiro de 2019, assim composta: Professores Doutores: Gisele Rodrigues da Silva (UFU); Priscilla Barbosa Ferreira Soares (UFU) Linda Wang (FOB-USP); Fabíola Galbiatti de Carvalho Carlo (UFJF) e orientador(a) do(a) candidato(a) **Veridiana Resende Novais Simamoto**

Iniciando os trabalhos o(a) presidente da mesa ***Dra. Veridiana Resende Novais Simamoto*** apresentou a Comissão Examinadora e o candidato(a), agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de argüição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos (às) examinadore (as), que passaram a argüir o(a) candidato(a). Finalizada a argüição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu os conceitos finais.

Em face do resultado obtido, a Banca Examinadora considerou o(a) candidato(a) (A)provado(a).

Esta defesa de Tese de Doutorado é parte dos requisitos necessários à obtenção do título de Doutor. O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos às 18 horas e 30 minutos. Foi lavrada a presente ata que após lida e achada conforme foi assinada eletronicamente pela Banca Examinadora.

Documento assinado eletronicamente por **Veridiana Resende Novais**, **Professor(a) do Magistério Superior**, em 25/02/2019, às 11:54, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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Dados Internacionais de Catalogação na Publicação (CIP) Sistema de Bibliotecas da UFU, MG, Brasil.

R696c 2019

Rodrigues, Renata Borges, 1989

Complicações da radioterapia no esmalte e dentina humanos = Complications of radiotherapy in human enamel and dentin [recurso eletrônico] : análise da adesão, composição química e propriedades mecânicas = adhesion analysis, chemical composition and mechanical properties / Renata Borges Rodrigues. - 2019.

Orientadora: Veridiana Resende Novais Simamoto.
Tese (Doutorado) - Universidade Federal de Uberlândia, Programa de Pós-Graduação em Odontologia.
Modo de acesso: Internet.
Disponível em: http://dx.doi.org/10.14393/ufu.te.2019.1222
Inclui bibliografia.
Inclui ilustrações.

1. Odontologia. 2. Esmalte dentário. 3. Dentina. 4. Radioterapia. I. Simamoto, Veridiana Resende Novais, 1979, (Orient.) II. Universidade Federal de Uberlândia. Programa de Pós-Graduação em Odontologia. III. Título.

CDU: 616.314

Angela Aparecida Vicentini Tzi Tziboy – CRB-6/947

DEDICATÓRIA

A Deus,

Obrigada Senhor pelo Dom da vida! Obrigada por ter estado ao meu lado em todos os momentos desta trajetória, iluminando meus caminhos e me livrando de todo o mal! Obrigada pelo Teu infinito amor que me fortalece todos os dias!!

Obrigada, obrigada, obrigada!!!

Ao Espirito Santo, Obrigada pela inspiração e força que recebo de Ti todos os dias!!!

Às minhas intercessoras... Nossa Senhora D'Abadia e Santa Apolônia,

Mãezinha querida, confio inteiramente na Sua intercessão e no seu cuidado. Agradeço imensamente por estar sempre me abençoando com seu amor de Mãe.

Obrigada Santa Apolônia, padroeira dos dentistas, a quem entrego minha profissão desde o primeiro dia da faculdade, obrigada pelo cuidado e por me inspirar cada dia a amar o que eu faço.

Aos meus Pais, Divina e José Mauriney,

Vocês que me deram a vida e me ensinaram a vivê-la com dignidade. Obrigada por toda a dedicação, todos os ensinamentos, todos os esforços e todo amor que sempre deram a mim. Obrigada pela paciência e compreensão com minhas ausências, obrigada por acreditarem nos meus sonhos e não medirem esforços para eu possa realizá-los, obrigada por acreditarem em mim!!!

Mãe, a senhora é o pilar da nossa família. A sua Fé me inspira. Obrigada por todo amor e dedicação, obrigada por viver cada momento dessa trajetória ao meu lado, obrigada por ser a minha melhor amiga, obrigada pela cumplicidade e amor incondicional!

Pai, o senhor é meu grande exemplo de determinação e força. Obrigada por sempre me ensinar que as coisas simples da vida são as mais importantes. Obrigada pelo amor e cuidado de sempre!

Amo vocês infinitamente!!!

Ao meu irmão, Fernando,

"Irmão é bom demais". Sempre falo isso quando me perguntam sobre família. Mas a verdade é que ter você como irmão é bom demais!!! Mesmo estando distante penso em você todos os dias e sinto a sua torcida constante por mim. Muito obrigada pelo carinho, pelo amor, pelo cuidado, pela cumplicidade, pela irmandade! Eu amo você!!! E obrigada por te me dado uma irmã de coração, minha querida cunhada **Renata,** a quem agradeço imensamente pela amizade, carinho e torcida!

Ao meu noivo, Jásio,

Você é o melhor companheiro de vida que Deus poderia ter me dado!! Obrigada pela amizade, pelo companheirismo, pela paciência e compreensão quando precisei desabafar, pelo cuidado que tem comigo, pela torcida constante, pelo amor!!! Obrigada por me fazer ver que eu tenho força nos momentos em que quis fraquejar.

Eu amo você!!

AGRADECIMENTO ESPECIAL

À Prof.ª Drª. Veridiana Resende Novais Simamoto,

Veri, agradeço a Deus por ter colocado você no meu caminho. Se estou aqui hoje foi porque você acreditou em mim e abriu as portas para que eu pudesse caminhar. Você tem o dom de ensinar! Muito obrigada por todo o aprendizado, por todas as oportunidades, pela orientação tão cuidadosa e pela confiança a mim depositada. Admiro muito a profissional que você é, a professora, a amiga, a mãe. Quem está próximo de você vê nos seus olhos que tudo que você faz irradia amor. E esse é o seu diferencial. O seu exemplo despertou em mim o interesse pela pesquisa e pela docência. Você sempre me incentiva a buscar novos conhecimentos e a me superar. Obrigada por abrir as portas da sua casa para mim. Obrigada pela amizade!! Você é muito mais que uma orientadora, é uma grande amiga, uma pessoa que faz parte da minha história e a quem eu sempre serei grata! Que Deus continue abençoando muito o seu trabalho, a sua família e a sua vida. Muito obrigada!!! Você é muito especial!!!

AGRADECIMENTOS

À ***Universidade Federal de Uberlândia, Faculdade de Odontologia e Programa de Pós-Graduação em Odontologia***, escola que me formou desde a graduação. Tenho muito orgulho de carregar o nome da UFU, FOUFU e PPGO comigo. Fica aqui meu reconhecimento e gratidão!!!

Aos ***Professores da FOUFU e PPGO,*** por todo aprendizado e crescimento pessoal e profissional

Ao Prof. Dr. Carlos José Soares, muito obrigada por todo aprendizado, orientação e amizade!!! Tenha certeza que o senhor inspira todos os alunos que passam por esse Programa!

Ao ***Prof. Paulo Simamoto***, muito obrigada pela amizade, por todos os ensinamentos e por sempre me acolher tão bem na sua casa.

Às ***Professoras da banca, Priscila, Gisele, Fabíola e Linda***, obrigada por terem aceitado o convite, contribuírem com o trabalho e participarem desse momento. ***Pri e Gisele,*** obrigada pela amizade e carinho que sempre senti todas as vezes que nos encontramos.

À ***Carol,*** grande amiga que a Faculdade me deu, presente de Deus na minha vida!!! Muito obrigada pela amizade sincera e verdadeira, pela torcida constante, pelos momentos de alegria, pela força que você sempre me deu nos momentos difíceis, pelas orações. Eu amo você!!!

À ***toda minha família,*** agradeço pelos momentos alegres vividos juntos, pela torcida constante e pelas orações.

À ***Luciara, Duda, Manu e Matheus***, muito obrigada pelo carinho, pela torcida e pelo aconchego da casa de vocês!!!

À ***Dona Solange, Jásio e Ana Paula*, ** obrigada por me fazer sentir tão acolhida na casa de vocês! Obrigada pelo carinho e por todos os momentos tão agradáveis que já passamos juntos. Vocês são muito especiais para mim!

Às amigas, ***Camila Peres, Camila Lopes e Lívia Zeola***, vocês são grandes presentes que a Pós-Graduação me deu. Tenho certeza que nossa amizade será para sempre!!! Muito obrigada por tudo!! Cada uma de vocês tem uma importância muito grande em minha vida!!!

À ***amiga Fernanda Castelo***. Fer, nossa amizade deve ser de outras vidas. Muito obrigada pelo carinho que sempre teve comigo, pela confiança, por ter aberto tantas portas para mim. Obrigada por tudo!!! Vc é uma amiga muito especial!!

À minha amiga de sempre ***Poliana***, obrigada por juntas provarmos que uma amizade verdadeira é maior que o tempo e a distância! Eu amo você!

À minha irmã de coração ***Vanessa Cotian***, muito obrigada pela amizade e torcida constante. Muito obrigada por sempre cuidar da nossa amizade e não deixar que a distância nos afaste. Eu amo você!!

À, ***Marininha,*** você esteve ao meu lado desde o primeiro dia da iniciação científica. Muito obrigada pela amizade, pela torcida e pelo carinho de sempre! Você é muito especial!

Ao ***Grupinho de 10***, meninas apesar da distância vocês estão sempre no meu coração. Muito obrigada pela amizade de todas vocês e pela torcida de sempre!!!

Aos amigos do grupo de pesquisa ***Novais VR,*** obrigada a cada um pela torcida e carinho que sempre senti em todos os momentos que estivemos juntos.

Ao amigo, ***Rafael Resende***. Rafa obrigada pela amizade e toda ajuda. Te desejo muito sucesso! Conte sempre comigo!

À Faculdade Pitágoras, em especial aos ***Professores e alunos do curso de Odontologia***, instituição que me acolheu tão bem. Onde tenho um imenso prazer em trabalhar. Onde estou aprendendo e vivenciado as dificuldades e a imensa satisfação em ser professor.

Aos colegas professores, obrigada pela amizade e parceria no dia a dia.

Aos meus alunos, obrigada por tudo que aprendo com vocês, profissional e pessoalmente, todos os dias.

Aos amigos da nossa ***turma de Doutorado e da Pós-Graduação***. Apesar de não estarmos sempre juntos esses 4 anos, todos tiveram um importante papel nessa caminhada de muito aprendizado. Desejo muito sucesso a todos!

Ao ***Sr. Advaldo,*** muito obrigada pela amizade, pelo carinho, pelos chocolates e pelas preocupações comigo! O senhor é uma pessoa com um coração enorme! Muito obrigada por tudo!

Aos ***Funcionários da FOUFU,*** em especial Graça e Brenda. Muito obrigada pela amizade e ajuda sempre que precisei.

Aos ***Técnicos do CPBio, Jonh Douglas, Eliete e Bruno***, obrigada por toda ajuda no laboratório.

Ao ***Setor de Radioterapia do Hospital de Clínicas da UFTM,*** muito obrigada pela parceria.

À ***Giovanna e Raquel,*** muito obrigada pela amizade e por todos os momentos alegres vividos juntas! Gi muito obrigada por todos os ensinamentos!

À ***CAPES,*** pelo apoio financeiro por meio da bolsa de Doutorado, que recebi por 2 anos..

À ***FAPEMIG,*** pelo apoio financeiro.

Ao ***CNPq***, pelo apoio financeiro.

Agradeço a todas as pessoas que tive a oportunidade de conviver e com elas aprender!!!

MUITO OBRIGADA!!!

"Não existem sonhos impossíveis para aqueles que realmente acreditam que o poder realizador reside no interior de cada ser humano. Sempre que alguém descobre esse poder, algo antes considerado impossível, se torna realidade."

Albert Einsten

SUMÁRIO

RESUMO

A Radioterapia (RT) é uma terapêutica eficaz no tratamento e controle de neoplasias malignas na região de cabeça e pescoço. No entanto, pode causar reações adversas em estruturas saudáveis que inevitavelmente participem do campo de irradiação. Uma das principais consequências quando a RT envolve a cavidade oral é a cárie relacionada à radiação (CRR). Seu tratamento é desafiador. Este trabalho envolveu 4 objetivos sequenciais: **Objetivo 1**: avaliar a literatura buscando condensar informações sobre alterações do esmalte e dentina após a radioterapia e sua influência na adesão a estes substratos. **Objetivo 2:** avaliar a resistência de união de um sistema adesivo (Scothbond - 3M, ESPE) por meio de ensaio de microcisalhamento, entre resina composta e esmalte irradiado, antes e após a realização da radioterapia *in vitro.* **Objetivo 3:** avaliar as alterações ocorridas na dentina da região cervical após a radioterapia *in vitro*. Para isso foi utilizada análise química por Espectroscopia Infravermelha Transformada de Fourier e Espectroscopia Raman, e análise mecânica por meio de Nanodureza e Módulo de Elasticidade. **Objetivo 4:** associar os resultados encontrados nos objetivos 1, 2 e 3 em um artigo com foco clínico, para comunicação aos cirurgiões-dentistas. Por meio da revisão de literatura, realizada no objetivo 1, pode-se concluir que a radioterapia causa alterações mecânicas e químicas no esmalte e na dentina, e essas alterações comprometem negativamente a resistência de união de restaurações adesivas. No entanto não há na literatura uma padronização sobre o melhor sistema adesivo a ser utilizado nessa situação clínica. O objetivo 2 comprova as conclusões do objetivo 1, ao mostrar que a radioterapia realizada previamente à restauração em esmalte ocasionou a formação de uma interface adesiva heterogênea e menores valores de resistência de união quando comparados a um grupo não irradiado e a um grupo restaurado previamente à radioterapia. O objetivo 3, buscando compreender alterações que contribuam para o acometimento da CRR na região cervical, mostrou que a dentina desta região tem sua composição química alterada e propriedades mecânicas diminuídas, contribuindo para a rápida progressão da CRR. Por fim, mostrou-se com o objetivo 4, a importância de o cirurgião dentista conhecer as alterações ocasionadas pela radioterapia nos tecidos dentais e cavidade oral, tomando consciência dos cuidados no tratamento restaurador de pacientes pós radioterapia.

Palavras-chaves: Análise Espectral Raman, Esmalte dentário, Dentina, Dureza, FTIR, Radioterapia, Resistência ao Cisalhamento

ABSTRACT

Radiotherapy (RT) is an effective therapy in the treatment and control of malignant neoplasms in the head and neck region. However, it can cause adverse reactions in healthy structures that inevitably participate in the irradiation field. One of the main consequences when RT involves the oral cavity is radiation-related caries (CRR). Your treatment is challenging. This work involved 4 sequential objectives: Objective 1: to evaluate the literature seeking to condense information on enamel and dentin alterations after radiotherapy and its influence on adhesion to these substrates. Objective 2: to evaluate the bond strength of an adhesive system (Scothbond -3M, ESPE) by means of a microcrystalline test, between composite resin and irradiated enamel, before and after in vitro radiotherapy. Objective 3: to evaluate changes in the dentin of the cervical region after in vitro radiotherapy. For this, chemical analysis was used by Fourier Transform Infrared Spectroscopy and Raman Spectroscopy, and mechanical analysis by means of Nanodureza and Modulus of Elasticity. Objective 4: to associate the results found in objectives 1, 2 and 3 in an article with clinical focus, for communication to dentists. Through the literature review, performed in objective 1, it can be concluded that radiotherapy causes mechanical and chemical changes in the enamel and dentin, and these alterations negatively affect the bond strength of adhesive restorations. However, there is no standardized literature on the best adhesive system to be used in this clinical situation. Objective 2 confirms the conclusions of objective 1, showing that the radiotherapy performed prior to enamel restoration caused the formation of a heterogeneous adhesive interface and lower union strength values when compared to a non-irradiated group and to a group restored before radiotherapy. Objective 3, seeking to understand changes that contribute to the involvement of RRC in the cervical region, showed that the dentin of this region has its chemical composition altered and its mechanical properties diminished, contributing to the rapid progression of CRR. Finally, it was shown with objective 4, the importance of the dental surgeon to know the changes caused by radiotherapy in dental tissues and oral cavity, becoming aware of the care in the restorative treatment of patients after radiotherapy.

Key words: Raman Spectral Analysis, Dental Enamel, Dentin, Hardness, FTIR, Radiotherapy, Shear Strength

INTRODUÇÃO E REFERENCIAL TEÓRICO

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

A qualidade de vida de pacientes com câncer na região de cabeça e pescoço que fazem tratamento com radioterapia pode ser drasticamente diminuída devido a numerosas complicações orais induzidas por meio da iradiação (De Felice et al., 2018). Câncer de cabeça e pescoço (CCP) constitui um grupo heterogêneo de neoplasias malignas, constituído por vários sítios anatômicos, incluindo a nasofaringe, seios paranasais, cavidade oral, orofaringe e laringe (De Felice et al., 2018). São frequentemente tratados com radioterapia, uma técnica que utiliza radiação ionizante e danifica de forma semi-seletiva material genético de células malignas vulneráveis, ou através da produção de radicais livres, levando a morte celular (De Felice et al., 2018).

No Brasil, segundo o Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), estimam-se 11.200 casos novos de câncer da cavidade oral em homens e 3.500 em mulheres para cada ano do biênio 2018-2019. Esses valores correspondem a um risco estimado de 10,86 casos novos a cada 100 mil homens, ocupando a quinta posição; e de 3,28 para cada 100 mil mulheres, sendo o 12º mais frequente entre todos os cânceres (INCA 2017). Não há norma ou padronização nas literaturas nacional e internacional sobre quais estruturas anatômicas compõem a sua definição, por isso de acordo com o INCA foram consideradas como neoplasias malignas de lábio e cavidade oral aquelas que tenham como localização primária os lábios, a cavidade oral, as glândulas salivares e a orofaringe (C00-C10) CID 10 (INCA 2016). Além do câncer oral, existem outros tipos de câncer que se localizam na região de cabeça e pescoço, e dentre eles, o câncer de laringe é o mais comum (INCA 2017). A estimativa são 6.390 casos novos de câncer de laringe em homens e 1.280 em mulheres para cada ano do biênio 2018-2019 (INCA 2017).

A radioterapia é uma forma terapêutica amplamente utilizada para o tratamento dessas neoplasias, de forma exclusiva ou associada à cirurgia e quimioterapia (Kielbassa et al., 2006; Lazarus et al., 2007; De Felice et al., 2018). De acordo com informações também fornecidas pelo Instituto Nacional do Câncer, a radioterapia é feita utilizando radiações ionizantes que podem ser eletromagnéticas ou corpusculares e que, carregam energia ao interagirem com os tecidos dando origem a elétrons rápidos que ionizam o meio e criam efeitos químicos como a hidrólise da água e a ruptura das cadeias de DNA. Durante o processo de ionização, os átomos são alterados, o que pode acarretar também alteração das estruturas das moléculas que os contém, assim, se a energia de excitação ultrapassar a energia de ligação entre os átomos pode ocorrer a quebra das ligações químicas e consequentemente alterações moleculares (INCA). A unidade internacionalmente utilizada para medir a quantidade de irradiação é Gray (Gy), que expressa a dose de radiação absorvida por qualquer material ou tecido humano. Quando a dose de radiação é elevada (vários Gy), muitas células dos tecidos que estão recebendo essa radiação podem não suportar as alterações e morrerem após tentativas de se dividir. As radiações ionizantes penetrantes podem induzir danos em diversas profundidades no organismo humano e com isso causar a morte celular. Por isso, são utilizados tratamentos com radiação para a terapia do câncer (INCA). Sua função é erradicar células cancerígenas para diminuição do tumor e posterior cirurgia, ou em alguns casos pode ser usada como tratamento exclusivo, e ainda pode ser utilizada como tratamento paliativo, em casos em que não há previsão de cura (De Felice 2018).

No geral, a dose total de radiação para tratamento de câncer de cabeça e pescoço varia de 30 a 72 Gy, dependendo do tipo de tumor e do seu volume. Com o objetivo de eliminar eficazmente as células tumorais e minimizar os efeitos colaterais ao tecido sadio, os protocolos convencionais de radioterapia fornecem a dose de radiação prescrita em múltiplas frações diárias (geralmente 2 Gy / fração), administradas por várias semanas. Durante o planejamento da radioterapia, é importante garantir uma adequada cobertura às células tumorais e minimizar o risco de toxicidade induzida aos tecidos saudáveis próximos. É fundamental uma definição precisa dos órgãos e estruturas em risco no plano de tratamento. Na região de cabeça e pescoço existe uma quantidade enorme de estruturas em risco e, por isso, muitas vezes não é possível respeitar todas as restrições de dose, especialmente em caso de doença avançada (Good & Harrington 2013).

Quando o campo da radioterapia envolve a cavidade bucal, muitas complicações podem ocorrer. Essas complicações são o resultado dos efeitos nocivos da radiação sobre glândulas salivares, mucosa oral, osso, dentes, musculatura mastigatória e as articulações temporomandibulares (Vissink et al., 2003; Jham & Freire., 2006). As consequências clínicas orais da radioterapia incluem mucosite, hipossalivação, perda de paladar, osteorradionecrose, trismo e cárie relacionada à radiação (Vissink et al., 2003;Jham & Freire., 2006). A intensidade destas reações irá depender do volume, do local irradiado, da dose total, do fracionamento da dose, da idade, das condições clínicas do paciente e dos tratamentos associados (Caccelli & Raport 2008).

É importante e necessário o acompanhamento clínico odontológico de pacientes submetidos à radioterapia na região de cabeça e pescoço antes, durante e após o tratamento (Kielbassa et al., 2006). Uma consequência conhecida são as cáries relacionadas a radiação que se desenvolvem rapidamente e são consideradas formas altamente destrutivas de cárie (Kielbassa et al., 2006, Gupta et al., 2015, Queiroz et al., 2019). Alterações nas glândulas salivares, que causam a hipossalivação, alterando o fluxo e a composição salivar, associado às alterações na composição e propriedades mecânicas da estrutura dentária, tornam os dentes altamente susceptíveis ao acometimento de lesões cariosas após a radioterapia (Jawad et al., 2015; Rodrigues et al., 2018; Miranda et al., 2018). Além disso, modificações induzidas no meio oral pela radiação, como alterações gustatórias e dietéticas levando a preferência por alimentos macios e ricos em carboidratos, queda no pH salivar com consequente alteração da flora oral e dificuldades de higienização tornam o meio oral desses pacientes altamente cariogênico (Kielbassa et al., 1997; Vissink et al., 2003; Walker et al., 2011; Jawad et al., 2015).

Muitos estudos mostram alterações no esmalte e na dentina após a radioterapia (Soares et al., 2010; Gonçalves et al., 2014; Lieshout & Bots 2014;Qing et al., 2015; Reed et al., 2015; Novais et al., 2016; Qing et al., 2016; Liang et al., 2016; Rodrigues et al., 2018; Lopes et al., 2018; Miranda et al., 2018, Velo et ., 2018; Lu et al., 2019). Os raios ionizantes da radioterapia causam a quebra da molécula de água, que corresponde a aproximadamente 3% do esmalte e 12% da dentina, gerando radicais livres de hidrogênio e peróxido de hidrogênio que são fortes reagentes de oxidação (Pioch et al., 1992). Ao agirem podem causar a desnaturação dos componentes orgânicos das estruturas dentais (Pioch et al., 1992). Para ambos os tecidos, essas alterações são dose-dependentes, ou seja, quanto maior a dose radioterápica mais evidentes são as alterações morfológicas e mecânicas (Gonçalves et al., 2014; Liang et al., 2016).

No esmalte observa-se alterações na matriz inorgânica e orgânica. Embora seja composto em 96% por cristais de hidroxiapatita (matriz inorgânica), 3% de água e aproximadamente apenas 1% de conteúdo proteico (Nanci & Tem Cate 2008), alterações na matriz orgânica comprometem o comportamento mecânico do esmalte (Santin et al., 2015). A região interprismática, onde se localiza a matriz orgânica, torna-se mais evidente e apresenta fissuras (Gonçalves et al., 2014; Madrid et al., 2017). Além disso alterações na matriz inorgânica alteram a cristalinidade do esmalte (Lopes et al., 2018), tornando-o um tecido mais friável e consequentemente enfraquecido mecanicamente (Santin et al., 2015).

A dentina madura é composta de aproximadamente 60% de material inorgânico (hidroxiapatita), 28% de material orgânico e 12% de água por peso (Fejerskov et al., 2005; Nanci & Ten Cate, 2008). Sua matriz orgânica consiste em 90% de colágeno (principalmente tipo I com pequenas quantidades dos tipos III e IV) com inclusões fracionais de proteínas não colágenas da matriz (Giannini et al., 2004). O colágeno tipo I age como suporte que acomoda e mantêm juntos os cristais de apatita nos orifícios e poros das fibrilas, alguns dos quais estão precipitados dentro da fina estrutura de hélice de colágeno. Com essa estrutura há sinergia entre matriz e apatita (Nanci &Ten Cate, 2008). A literatura mostra que a radioterapia é capaz de alterar a matriz orgânica da dentina, alterando sua estrutura e causando a fragmentação das fibras colágenas (Gonçalves et al., 2014; Velo et al., 2018; Miranda et al., 2018; Lu et al., 2019). Também já foi mostrado que há alterações na porção inorgânica com troca de íons fosfato-carbonato na hidroxiapatita (Miranda et al., 2018), com consequente diminuição da interação mineral-orgânica (Rodrigues et al., 2018). Tais alterações comprometem as propriedades mecânicas da dentina irradiada.

Outra região que tem sido foco dos pesquisadores é a junção esmalte dentina (JED). Estudos mostram que esta é uma região com alta instabilidade pós radioterapia (Mc Guire et al., 2014; Queiroz et al.,2019). Essa instabilidade contribui para a perda do esmalte que pode ocorrer em um processo chamado delaminação (Reed et al., 2015). Nesse processo, forças de cisalhamento e alterações químicas fazem com que o esmalte se descole da dentina, na região da JED, especialmente na região cervical dos dentes, deixando a dentina subjacente exposta (Walker et al., 2011). Este é um dos fatores que contribue para o acometimento da cárie relacionada à radiação. Após a radioterapia, o ambiente oral torna-se bastante cariogênico devido à hipossalivação e xerostomia e, associado a isso, o esmalte e a dentina apresentam suas propriedades alteradas. Essa somatória de fatores faz com que a progressão desse tipo de cárie seja rápida e seu tratamento desafiador.

A restauração de cáries relacionadas à radiação pode ser extremamente desgastante para pacientes e cirurgiões-dentistas (Vissink et al., 2003; Gupta et al., 2015). O sucesso clínico de restaurações adesivas depende da efetividade e durabilidade da interface de união, o que torna necessário o conhecimento sobre os substratos dentários nos quais os sistemas adesivos serão aplicados e o mecanismo pelo qual ocorre esta união (Martins et al., 2008). Autores demonstraram a ineficiência da adesão entre a restauração e o substrato dentário de dentes irradiados, principalmente após altas doses de irradiação (Naves et al., 2012; Yadav & Yadav 2013; Rodrigues el al., 2018). Esse processo de adesão de materiais restauradores ao esmalte e à dentina ocorre por mecanismo básico no qual há um processo de troca, que envolve a substituição dos minerais removidos dos tecidos dentais, por monômeros resinosos, que se infiltram e são polimerizados nas porosidades criadas, promovendo adesão micromecânica (Conceição 2007). Nesse sentido, não há protocolos restauradores bem estabelecidos na literatura que orientem o profissional sobre a melhor forma de tratar esses pacientes.

Diante desse cenário, a compreensão de como as alterações ocorridas no esmalte e dentina após a radioterapia podem interferir na adesão a estes tecidos torna-se importante. Além disso, a busca por respostas que aumentem a compreensão sobre o desenvolvimento da cárie relacionada à radiação e, a orientação de profissionais quanto ao seu tratamento tornam-se de grande relevância.

OBJETIVOS

2. OBJETIVOS

Objetivo geral

O objetivo geral deste trabalho foi avaliar as complicações que a radioterapia na região de cabeça e pescoço pode gerar no esmalte e na dentina, assim como sua influência nos procedimentos restauradores adesivos. Objetivou-se ainda avaliar as alterações químicas e mecânicas na dentina da região que mais é acometida pela cárie relacionada a radiação, a dentina cervical.

Objetivos específicos

Objetivo específico 1

Capítulo 1 - Complications of radiotherapy in dental bonding– a literature review

Este objetivo específico avaliou a literatura buscando condensar informações sobre alterações do esmalte e dentina após a radioterapia e sua influência na adesão a estes substratos.

Objetivo específico 2

Capítulo 2 - Effect of radiotherapy on the bond strength of composite resin to human enamel

Este objetivo específico avaliou a resistência de união entre resina composta e esmalte irradiado, antes e após a realização da radioterapia *in vitro.*

Objetivo específico 3

Capítulo 3 - Impact of radiotherapy in chemical composition and mechanics properties in the cervical human dentin

Este objetivo específico avaliou as alterações ocorridas na dentina da região cervical após a radioterapia *in vitro*.

Objetivo específico 4

Capítulo 4 - Manejo odontológico de pacientes oncológicos de cabeça e pescoço: evidência científica com ênfase clínica na cárie relacionada à radiação

O objetivo deste capítulo foi discorrer sobre os resultados encontrados nos capítulos 1, 2 e 3 compilados em um artigo nacional, com foco clínico, para comunicação aos cirurgiões-dentistas brasileiros.

CAPÍTULOS

3. CAPÍTULOS

3.1 Capítulo 1

Complications of radiotherapy in dental bonding– a literature review

Artigo a ser submetido para o periódico *Journal of Cancer Research and Clinical Oncology*

Complications of radiotherapy in dental bonding– a literature review

Abstract

Purpose: Radiotherapy is a widely used treatment for head and neck cancers. Patients undergoing and even after radiotherapy can develop oral complications, such as mucositis, candidiasis, hyposalivation followed by xerostomia, trismus, osteoradionecrosis and radiation-related caries. Radiation-related caries is considered a chronic sign of irradiation, being a highly destructive and rapidly progressive caries. It has a multifactorial origin, associating direct side effects (changes in enamel, dentin and dentin-enamel junction) to indirect side effects (hyposalivation, modifications in oral biofilms and saliva, hygiene limitations and more cariogenic diet - due to the painful symptoms of mucositis). In this manuscript, we discuss the importance of the biomechanical and morphological dental alterations associated to the radiation-related caries and the caries clinical aspects. Focus was given on the restorative treatment once the altered dental substrate could compromise bonding of adhesive materials.

Methods: The relevant literature from PubMed is reviewed in this study. The search terms were "radiotherapy", "adhesion", "bond strength", "enamel" and "dentin". There was no publication year limitation.

Results: Twelve accession articles were found that were included in this review. Nine evaluated adhesion to dentin, two evaluated adhesion to enamel and one article evaluated adhesion to the two substrates. There is no standardization regarding the radiotherapeutic dose neither adhesive systems used.

Conclusion: It was concluded that there is not yet an optimal restorative protocol for this clinical situation. The literature is still controversial but most studies show that there is a compromised bond strength of adhesive restorations after radiotherapy.

Keywords: Radiotherapy; Dental enamel; Dentin; Dental bonding.

Introduction

Head and Neck Cancer is a heterogeneous group of malignancies, consisting of various anatomic sites, including nasopharynx, paranasal sinuses, oral cavity, oropharynx, hypopharynx and larynx (De Felice and Tombolini 2018). Approximately 650.000 head and neck new cancer cases are diagnosed every year worldwide (Torre et al. 2015). This type of neoplasm can be treated with different methods, like surgery, irradiation, chemotherapy or a combination of these treatment modalities (Marur and Forastiere 2016). In general, a widely used treatment is radiotherapy, cause presents better anatomic and functional preservation with highly effective for tumor control (Tschiesner 2012). However, oral complications in the irradiated area are generally observed (Lieshout and Bots 2014). The toxicity of irradiation generates negative effects on healthy tissues located in the radiation field, as in salivary glands, soft tissues, bones and teeth (Kielbassa et al. 2006; Ray-Chaudhuri et al. 2013; Rodrigues et al. 2018).

Oral manifestations of head and neck radiotherapy include mucositis, candidiasis, hyposalivation followed by xerostomia, trismus, osteoradionecrosis and radiation-related caries (Goncalves et al. 2014; Vissink et al. 2003). The latter is considered a late or chronic sign of irradiation therapy. Therefore, radiation-related caries is a complex and destructive disease with a high potential for progression, and usually affects the cervical region of the teeth, which turns to brownish color (Galetti et al. 2014; Goncalves et al. 2014; Silva et al. 2009). Studies have shown their multifactorial origin, in which it is associated with direct side effects (changes in the properties of enamel, dentin and amelodentin junction), as well as indirect side effects (hyposalivation, modifications in oral biofilms, altered composition of saliva, hygiene limitations and diet more cariogenic - due to the painful symptoms of mucositis (Deng et al. 2015; Goncalves et al. 2014; Rodrigues et al. 2018).

In recent years, many studies have been devoted to explore the changes that radiotherapy can cause in dental structures and their consequences in the management and treatment of patients with head and neck cancer (Beech et al. 2014; de Miranda et al. 2018; Lopes et al. 2018; Novais et al. 2016; Rodrigues et al. 2018). It is already understood that occurs the formation of free radicals of hydrogen and hydrogen peroxide during the irradiation process (Pioch et al. 1992). It is believed that such radicals act as a strong oxidant that can cause denaturation of the organic components of enamel and dentin, thus altering their properties (de Barros da Cunha et al. 2017; Franzel et al. 2006; Goncalves et al. 2014; Kielbassa et al. 2006; Lieshout and Bots 2014; Soares et al. 2010; Velo et al. 2018). Once the organic matrix is damaged, its bonds with the apatite crystals are also compromised, which leads to a reduction of the organic-mineral interaction and consequent tissue fragility (Franzel et al. 2006).

The alteration of dental structure compromises the longevity of adhesive restorative treatments in irradiated enamel and dentin, since the formation of an ideal hybrid layer depends on the perfect micromechanical bonding between the adhesive system and the dental substrate (Rodrigues et al. 2018). This consequence represents a great challenge for restorative dentistry in patients submitted to head and neck radiotherapy. The literature is still controversial regarding the construction of protocols that establish the most suitable restorative techniques and materials for irradiated teeth. The scarcity of specific studies reinforces the importance of new research that guides an effective and long-lasting plan of restorative treatment.

In this manuscript, we will review and discuss the current literature offering information about the changes occurring in irradiated dental structures and their consequences on the bond strength of adhesive procedures. This information may guide new studies establishing an adequate restorative protocol for irradiated patients in the head and neck region.

Effects of radiotherapy in the enamel and dentin

Biomechanical changes in enamel

Dental enamel is a highly rigid and structurally organized tissue in a series of prisms rich in hydroxyapatite (mineral content) (Bulucu et al. 2006; Yadav and Yadav 2013). The interprismatic region contains the organic matrix that is present in lesser amounts (Cui and Ge 2007). Its composition, as well as that of dentin, is subject to damage from irradiation therapy. The morphological alterations in the enamel structure, after radiotherapy, is evidenced of the interprismatic portion, due to the higher concentration of protein and water. Although present in a small quantity, the organic matrix plays a fundamental role in the maintenance of the mechanical characteristics of the enamel (Santin et al. 2015), guaranteeing dissipation of forces between the prisms when the enamel receives some impact (Santin et al. 2015). Its degradation by radiotherapy makes the tissue less resistant to fracture and more friable (Santin et al. 2015; Seyedmahmoud et al. 2018). There is a decrease in the absorption and dissipation of forces (Santin et al. 2015) with reduction of microhardness (Seyedmahmoud et al. 2018). After 30 Gy of radiotherapeutic dose, it is possible to observe alteration in the interprismatic region of the enamel (Goncalves et al. 2014; Madrid Troconis et al. 2017). In addition, changes in the inorganic matrix of irradiated enamel was also shown by Lopes et al. 2018, with an increase in the carbonate content, causing crystalline deformation and making the enamel more soluble in acid, and consequently more susceptible to caries. This change had also been shown by Pioch et al. 1991. Deteriorating changes in enamel wear behavior also makes the tissue more susceptible to caries (Qing et al. 2016).

Another important region and that is directly related to the fragility of enamel post irradiation is the dentin-enamel junction (DEJ). The DEJ plays an important role in the mechanical stability of the teeth and, together with the internal enamel, near to DEJ, inhibit the crack propagation and present greater fracture toughness (Imbeni et al. 2005). However, in irradiated patients, it is common to observe clinically the complete enamel loss, suggesting DEJ instability (McGuire et al. 2014; Reed et al. 2015; Walker et al. 2011). Studies to understand this instability have shown a reduction of type IV collagen in teeth submitted to radiotherapy (McGuire et al. 2014). Considering that this collagen has a supporting function, its decrease in DEJ may justifies the fragility of this region (McGuire et al. 2014). Under normal occlusal loads, the DEJ is destabilized in caries resistant regions, like incisal / occlusal and cervical regions. This probably results in the formation of microcracks of enamel or slit formation in DEJ, leading to extreme bacterial colonization and severe caries. All these biomechanical changes lead to tooth enamel loss from dentin in a process called delamination, unique pattern of destruction associated with radiotherapy (McGuire et al. 2014; Reed et al. 2015; Thiagarajan et al. 2017).

Biomechanical changes in dentin

Dentin is an avascular and mineralized tissue composed of 70% inorganic matter, 18% organic matrix and 12% water (Mjor 1972). For presenting large amount of organic matrix and water, is a very affected tissue by the direct effects of radiotherapy (de Miranda et al. 2018; Novais et al. 2016; Qing et al. 2016; Rodrigues et al. 2018). These variations occur because free radicals produced during irradiation, in the presence of water, alter the chemical bonds of the collagen structure, disrupting the organic matrix of the dentin (Goncalves et al. 2014; Rodrigues et al. 2018; Soares et al. 2010). Dentin has crystals of biological hydroxyapatite formed in the middle of the collagen matrix. These crystals are smaller and have a less perfect structure when compared to the enamel crystals. In addition, the mineral portion of the dentin has a higher carbonate content (Goldberg et al. 2011). This structure gives dentin a characteristic of greater solubility, and these differences between dental substrates are important to make the tooth an organ that can resist the processes of demineralization and wear (Mjor 1972; Qing et al. 2016; Reed et al. 2015; Urabe et al. 2000). That said, any change in this dynamic makes the tooth more susceptible to the processes of caries deterioration.

Radiotherapy alters the chemical composition and mechanical properties of dentin (Campi et al. 2019; de Miranda et al. 2018; Rodrigues et al. 2018; Velo et al. 2018). Studies with specific methodologies show that the radiation can influence the exchange of phosphate-carbonate ions in the hydroxyapatite, as well as decrease the rate of mineralization of the hydroxyapatite, making the dentin more soluble (Velo et al. 2018). Another study, when evaluating the crystallinity and composition of the inorganic portion of irradiated dentin, showed that gamma irradiation induced the formation of larger crystals and greater amount of carbonate, negatively reflecting the wear behavior and the presence of larger and deeper cracks, parallel to the dentinal tubules (Qing et al. 2016). Besides that, the irradiation causes a disruption of the organic matrix of the dentin and a reduction of its cohesive strength (Soares et al. 2011).

These changes in the chemical composition directly reflect the mechanical behavior (Miguez et al. 2004). After a cumulative irradiation dose, it is possible to observe a disorganization of the dentin structure and a decrease in its modulus of elasticity (Rodrigues et al. 2018). Dentin structure cracks, dentinal tubule obliteration and collagen fiber fragmentation leads the reduction of microhardness in irradiated dentin when compared to non-irradiated dentin (Goncalves et al. 2014). Such changes make the dentin more susceptible to caries involvement and progression. This is because this tissue is mechanically fragile and chemically compromised.

Table 1 shows studies that evaluated the effect of irradiation used for radiotherapy on enamel and dentin and its main results.

Table 1 – Summarized results and conclusions of dental hard tissues alterations after head and neck radiotherapy based on in vitro, in situ and in vivo studies.

Author	***Irradiation dosage and type of study***	***Dental structure and analysis performed***	***Results and conclusions***
Pioch et al. 1992	70 Gy *In vitro*	Shear of deninoenamel junction of bovine incisors	Destruction of the organic matrix by radiolysis Reduction stability dentinoenamel junction
Kielbassa et al. 1997	60 Gy *In vitro*	Knoop hardness of dentin of bovine incisors	Reduction of dentin hardness
Kielbassa et al. 2002	60 Gy *In vitro*	Knoop hardness of dentin of bovine incisors	Reduction of dentin hardness Demineralization can be hampered by regular fluoride application in irradiated dentin. However, due to the considerable irradiation effect, this benefit might be negligible.
Franzel et al. 2006	60 Gy *In vitro*	Nanoindentation of human enamel and dentin	Reduction of mechanical properties (microhardness and elastic modulus)

Soares et al. 2010	60 Gy *In vitro*	Ultimate tensile strength of human enamel and dentin	Reduction of ultimate tensile strength of enamel and dentin Irradiation is more harmful to organic components.
Soares et al. 2011	60 Gy *In vitro*	Ultimate tensile strength of human enamel and dentin with chlorhexidine and fluoride mouthwash	Structural alterations, in both substrates, were detected by scanning electron microscopy analysis. Mouthwash with 0.12% chlorhexidine partially prevented the damage to the mechanical properties of the irradiated crown dentin, whereas the 0.05% sodium-fluoride-irradiated enamel showed UTS similar to that of non-irradiated enamel.
Gonçalves et al. 2014	10, 20, 30, 40, 50 e 60 Gy	Longitudinal evaluation of microhardness and MEV of human enamel and dentin, every 10 Gy of radiation	Increase in surface enamel microhardness Reduction in medium dentin microhardness Morphological changes in the enamel and dentin

			The increase of the cumulative radiation doses resulted in progressive micro morphological alterations of enamel and dentin structures. In enamel, the interprismatic portion became more evident, while fissures, dentinal tubules obliteration and fragmentation of collagen fibers were observed in dentin.
McGuire et al. 2014	70 Gy – In vitro >60 Gy In vivo	Confocal Immunofluorescent Staining SDS-PAGE and Western Blotting	Confocal microscopy revealed that immunostained type IV collagen was restricted to the 5- to 10-µm-wide optical DEJ, while collagenase treatment or previous in vivo tooth-level exposure to > 60 Gray irradiation severely reduced immunoreactivity

de Siqueira Mellara et al. 2014	10, 20, 30, 40, 50 and 60 Gy In vitro	Microhardness and morphology (SEM) of enamel and dentin of deciduous teeth	The enamel microhardness increased at a dose of 60 Gy, whereas the value of the dentin microhardness did not change. A progressive disruption of enamel and dentin morphology was found with the increased radiation dose
Reed et al. 2015	70 Gy *In vitro*	Nanoindentation and Raman Spectroscopy of human enamel and dentin	Increase in the stiffness of enamel and dentin near the dentin enamel junction Decrease in the dentin and enamel protein content.
Qing et al. 2015	70 Gy *In vitro*	Nanoscratch, X-ray diffraction, microhardness and FTIR of human enamel	Irradiation had a direct deleterious effect on the wear behaviour of human tooth enamel. An inferior nanoscratch resistance was observed.

			Variation of wear behaviours was closely related to changes in the crystallography, chemical composition and microhardess of the enamel.
Novais et al. 2016	60 Gy In vitro	Microhardness and flexural strength of human and bovine root dentin	The endodontic treatment and radiotherapy resulted in significantly lower KHN irrespective of tooth origin
Qing et al. 2016	70 Gy *In vitro*	Nanoscratch, SEM, X-ray diffraction and FTIR of human dentin	Irradiation affected directly the wear behavior of dentin, accompanied by the alterations in crystallography, chemical composition, and surface microhardness.
de Sa Ferreira et al. 2016	54 Gy In vitro	Microenergy-dispersive X-ray fluorescence and Fourier transform Raman spectroscopy before	RT application at a therapeutic dose reduced the organic content of the deciduous enamel.

		and after a pH cycling process in deciduous teeth enamel	
Liang et al. 2016	30, 50 e 70 Gy *In vitro*	Mechanical properties (elastic modulus, nanohardness and friction coefficient) of human enamel and dentin	Radiotherapy caused nano-mechanical changes in dentin and enamel that were dose related. The key doses were 30-50 Gy and the key time points occurred during the 15th-25th days of treatment, which is when application of measures to prevent radiation caries should be considered.
da Cunha et al. 2017	20, 40, and 70 Gy In vitro	Microhardness, superficial morphology, and mineral components of human enamel	Decrease in microhardness values only in the cervical enamel, regardless of the radiation dose used; no morphological or mineral change was observed.
Thiagarajan et al. 2017	Finite elements analisys	Stress analysis of enamel	Failure occurs at the inner enamel/DEJ interface due to extremely high tensile

			and maximum shear stresses in in vivo irradiated teeth
Rodrigues et al. 2018	72 Gy *In vitro*	Chemical composition (FTIR), SEM, microhardness (VHN) and elastic modulus (E) of human dentin	Alteration of the absorption bands in FTIR, SEM images showed a disorganization of the dentin structure. Mechanical properties were changed with increased VHN and decreased E
Lopes et al. 2018	70 Gy *In vitro*	Chemical composition (FTIR), SEM, microhardness (VHN) and elastic modulus (E) of human enamel irradiated with and without fluoride use	Fluoride reduced mineral loss and maintained the outer morphology of irradiated enamel. However, it was not as effective in preserving the mechanical properties of enamel. Radiotherapy altered the enamel's elastic modulus and its chemical composition.
de Miranda et al. 2018	72 Gy *In vivo* -	Chemical composition (FTIR	Radiotherapy altered the chemical

	Teeth extracted from patients submitted to radiotherapy	and Raman) of crown or root dentin	composition of human dentin. The exchange of phosphate-carbonate ions in the hydroxyapatite and higher concentration of organic components was found after radiotherapy.
Velo et al. 2018	70 Gy *In vitro*	Surface hardness, energy dispersive X-ray spectroscopy, and X-ray diffraction of human root dentin	Radiation exposure changes the composition and structure of human root dentin, which detrimentally affect its hardness.
Seyedmahmoud et al. 2018	*In vitro* (70 Gy) and *In vivo* (Teeth extracted from patients submitted to radiotherapy with doses >60 Gy)	Microhardness and Morphology and structural characterization (SEM) of human enamel	*In vitro* and *In vivo* irradiation alters enamel microhardness. Indentation pattern differences suggest that enamel may become more brittle following *in vitro* and *in vivo* irradiation.
Campi et al. 2018	60 Gy *In vitro*	Chemical composition by Raman	The radiotherapy was able to cause changes in *v4*PO_4^{3-},

		Spectroscopy of human root dentin	carbonate, and amide III peaks of root dentin
Queiroz et al. 2018	60 Gy *In vitro*	Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in permanent human teeth	Irradiation increased metalloproteinase activity in all regions of the DEJ.
Lu et al. 2019	30 e 60 Gy	Vickers microhardness tester and atomic force microscopy of human enamel and dentin	Radiation could directly alter the mechanical properties, micro-morphology, crystal properties, and chemical composition of dental hard tissue. The early destruction of DEJ-adjacent enamel, combined with decreased crystallinity of enamel under radiation exposure, may be related to the formation of characteristic radiation caries.

Radiation-related caries

Radiation-related caries is a complex, highly destructive and multifactorial disease (Baker 1982; Jongebloed et al. 1988; Kielbassa et al. 2006). This radiogenic dental damage results from the association of direct damage to enamel and dentin (Dreizen et al. 1977b; Kielbassa et al. 2006) previously reported, with indirect effects. Such indirect effects include quantitative and qualitative changes in saliva; difficulty of hygienization, due to painful symptoms by mucositis and consequent increased cariogenic diet (Andrews and Griffiths 2001; Deng et al. 2015; Gupta et al. 2015; Meurman and Gronroos 2010). In this way, the oral environment of a patient becomes highly cariogenic. Post-radiotherapy lesions are different than caries in non-irradiated patients. The lesions develop with initial loss of enamel near the (DEJ) leading to partial to total enamel delamination resulting in exposed dentin that is more vulnerable to subsequent decay (Gupta et al. 2015; Queiroz et al. 2018; Walker et al. 2011).

In addition to the changes in chemical composition and mechanical properties already mentioned, a recent study showed that there is an increased expression of gelatinases in enamel and dentin (MMP-2, MMP-9) (Queiroz et al. 2018). As irradiation can cause lytic changes in collagen polypeptides (Strup-Perrot et al. 2006), it is also possible that stimulates the activation of MMPs in exposed teeth (McGuire et al. 2014). Metalloproteinases (MMPs) are enzymes that are part of a large family of endopeptidases responsible for the remodeling of extracellular matrix components (Bourd-Boittin et al. 2005; Sahlberg et al. 1999). Present in the enamel and dentin organic matrix, gelatinases (MMP-2 and MMP-9) are collagenolytic; that is, they have the ability to degrade collagen (Bourd-Boittin et al. 2005; Sahlberg et al. 1999). Its greater expression in irradiated teeth is closer to the DEJ, and this may be related to the instability of the junction, causing the delamination of the enamel and later the rapid evolution of the caries lesion in the dentin (Queiroz et al. 2018).

Clinically, radiation-related caries differs from classic caries in terms of location, appearance and progression. While typical dental caries occurs in pits and fissures and in the proximal areas between the teeth, post-radiotherapy dental lesions tend to occur in the cervical (junction between crown and root), cuspid and incisal regions. These are sites exposed to the occlusal load (incisal / cuspid) and flexural (cervical) (Walker et al. 2011). They are brownish in color, and due to changes caused by radiotherapy, the restorative treatment of these lesions becomes challenging.

The management of radiation caries includes the preventive measures and its treatment. Dental examination and full mouth radiographs should be done before the start of radiotherapy. Besides, restoration of carious lesion, endodontic therapy, and recontouring of restorations should be done to prevent any future complications (Gupta et al. 2015). The patients have to be instructed about good oral hygiene care (including flossing and the use of interdental brushes for difficult areas to access with normal toothbrushes) (Gupta et al. 2015).

Added to this oral care, during and after radiotherapy, the use of topical fluoride by the patient is fundamental. Topical application of 1.0% sodium fluoride gel daily is recommended to reduce the occurrence of radiation-related caries (Dreizen et al. 1977a; Epstein et al. 1998). It is important to note that the fluoride content of 1% of prescription products is much higher than over-the-counter toothpaste with 0.15% fluoride. As for the prescribed sodium fluoride gels that are used after radiotherapy, neutral pH products are preferred to acidified sodium fluoride which may irritate the oral mucosa (Walker et al. 2011). In addition to the use of fluoride, another strategy is the use of remineralizing dentifrices (which also provide soluble calcium and phosphate ions) that can also prevent root caries in irradiated patients (Hay and Thomson 2002; Papas et al. 2008).

After radiotherapy is completed, follow-up visits should be scheduled frequently for patients. Unfortunately, not all patients collaborate and often even under supervision, it is not always possible to prevent the development of radiation-related caries. If any carious lesion is detected, it should be restored immediately. The restoration of these caries can be extremely challenging due to technical issues and the selection of the most appropriate restorative material (Gupta et al. 2015). The ideal restorative material for such cases should demonstrate adequate adhesion, prevent secondary caries and resist dehydration and acid erosion (Gupta et al. 2015). Unfortunately, no material performs very well all these features. In addition, radiation-induced changes in enamel and dentin may compromise the bonding of adhesive materials, leading to clinical failure in restorative treatment (Gupta et al. 2015).

Adhesion to dental tissues

The Table 2 presents some of the main studies about adhesion in irradiated teeth, enumerating the methods of analysis, radiation dosage, the adhesive systems and the restorative material used, as well as the dental structures analyzed in the researches.

Table 2. Main methodological aspects and conclusions of the included studies on resistance of adhesive systems to irradiated dental substrates

Study	**Methods of Analyzes**	**Radiation Dosage**	**Adhesives Systems and Restorative Material**	**Dental Structure**	**Results**
Gernhardt et al. 2001	Microtensile Bond Strength	60 Gy	Scotchbond (3M Dental Products, Loughborough, UK.); Solobond Plus (Voco, Cuxhaven, Germany.);	Dentin	There was no significant difference in the tensile strength of the irradiated and control groups

			Prime&Bond 2.1 (DeTrey Dentsply, Dreieich, Germany.) Syntac (Vivadent, Schaan, Lichtenstein)		
Bulucu et al. 2006	Shear bond strength	60 Gy	Prime & Bond NT (Dentsply/ Caulk, Milford, DE, USA) Clearfil SE Bond (Kuraray Medical, Osaka, Japan)	Dentin	Irradiation significantly affected adhesion of composite to dentin in groups with Prime and Bond NT irradiated The group that received radiation before the restorative procedure showed statistically lower bond strength
Biscaro et al. 2009	Microshear bond strength	5Gy, 35 Gy e 70 Gy	Single Bond 2 (3M ESPE)	Dentin	Bond strength results were dose and material dependent

			Clearfil SE Bond (Kuraray) Adper Prompt (3M ESPE)		Single-step self-etching showed lower binding strength after irradiation
Naves et al. 2012	Microtensile bond strength	60 Gy	Adper Single Bond (3M ESPE)	Enamel and Dentin	Radiotherapy applied before restoration significantly reduced the bond strength to both substrates
Yadav et al. 2013	Microtensile bond strength	60 Gy	Single Bond (3M ESPE)	Dentin	Irradiation before tooth preparation deteriorated the microtensile bond strength.
Galetti et al. 2014	Microtensile bond strength	60 – 70 Gy (in vivo)	Single Bond 2 (3M ESPE) Easy Bond (3M ESPE) Clearfil SE Bond (Kuraray)	Dentin	Head and neck radiotherapy did not affect dentin bond strength for the adhesive materials tested

Santin et al. 2015	Microshear bond strength	60 Gy	Transbond XT (3M)	Enamel	Enamel fragments subjected to radiation had lower strength than nonirradiated samples Radiation decreased tooth enamel strength, and the specimens treated with radiotherapy had higher frequencies of adhesive failure
Bernard et al. 2015	Microtensile bond strength	50 Gy (in vivo)	OptibondFL, KerrFrance,Cr´eteil,France OptibondXTR, KerrFrance,Cr´eteil,France	Dentin	Radiotherapy had a significant detrimental effect on bond strength to human dentin
da Cunha et al. 2016	Microshear bond strength	20, 40 and 70 Gy	Adper Single Bond 2 (3M ESPE) Universal Single Bond (3M ESPE)	Enamel and Dentin	Radiotherapy did not affect the bond strengths of the adhesives to either enamel or dentin In dentin, the Universal Single

					Bond adhesive system showed higher bond strength values when compared with the Adper Single Bond adhesive system
Freitas Soares et al. 2016	Microtensile bond strength	60 Gy	Adper Scotchbond MP(3M ESPE) Clearfil SE Bond (Kuraray)		Composite restoration procedure should be done before radiotherapy, regardless of the adhesive system used
Rodrigues et al. 2018	Microtensile bond strength	72 Gy	Scotchbond Multi-Purpose (3M ESPE)	Dentin	The microtensile bond strength was affected by the period of radiotherapy and restoration (before or after).
Santin et al. 2018	Shear bond strenght	60 Gy	Transbond XT composite resin	Enamel	No statistically significant difference among

	Fuji Ortho LC resin-modified glass ionomer cement Ketac Cem Easymix conventional glass ionomer cement	non-irradiated and irradiated groups

The number of specific adhesion studies on irradiated teeth is still scarce. Within the studies evaluated, three studies have shown that radiotherapy does not affect dentin adhesion (da Cunha et al. 2016; Galetti et al. 2014; Gernhardt et al. 2001), and one to enamel (Santin et al. 2018). Eight show a decrease of the adhesive resistance between restorative material and dental substrate (Bernard et al. 2015; Biscaro et al. 2009; Bulucu et al. 2006; Freitas Soares et al. 2016; Naves et al. 2012; Santin et al. 2015; Yadav and Yadav 2013). Of these eight studies, six evaluated adhesion in dentin (Bernard et al. 2015; Biscaro et al. 2009; Bulucu et al. 2006; Freitas Soares et al. 2016; Rodrigues et al. 2018; Santin et al. 2015; Yadav and Yadav 2013), one evaluated enamel and dentin (Naves et al. 2012) and one evaluated bond strength of irradiated enamel only (Santin et al. 2015). This issue can be justified by the fact that, clinically, it is difficult to find cases of restorative treatment in enamel only, due to the instability of the DEJ in irradiated teeth, may leading to loss of enamel after radiotherapy (Reed et al. 2015). Another explanation is the highly invasive and rapidly evolving character of the radiated-related caries, which rapidly reaches the dentin substrate (Baker 1982; Jongebloed et al. 1988; Kielbassa et al. 2006). Even so, it is important to perform enamel studies, considering that the margins of the restoration are almost always enamel. The studies show some methodological variations that may justify the difference in results.

It is observed that most of the articles were in vitro studies that controlled the irradiation doses applied in their samples, except for two (Bernard et al. 2015; Galetti et al. 2014) that used *in vivo* irradiated teeth with indication of extraction. The use of teeth extracted from patients has the advantage of being more trustworthy, considering that the teeth go through other challenges present in the oral cavity, as hyposalivation and cariogenic diet. However, it is difficult to standardize. Considering that adhesion tests require specific care to the reliability of their results, the use of *in vitro* irradiation allows a greater control of possible factors that may influence the results (Braga et al. 2018). Regarding this dose, most of the studies evaluated a total amount of 60Gy, with the minimum reported dose being 50 Gy (Bernard et al. 2015) and the maximum being 72 Gy (Rodrigues et al. 2018). This data is important because the literature indicates that teeth exposed to radiation between 30-60 Gy are 3 times more likely to suffer moderate and severe damage when compared to non-irradiated teeth. Radiation doses greater than 60Gy increase this probability by up to 10 times (Walker et al. 2011). And the main fact associated with alteration of adhesive resistance after radiotherapy is the alteration of the structure (Rodrigues et al. 2018). Generally, the higher the radiation dose, the lower the bond strength (Biscaro et al. 2009).

Considering that adhesion quality is directly related the efficiency of penetration of monomers in interfibrillar spaces, a compromised interaction between adhesive system and enamel and / or dentin, will compromise the efficiency and effectiveness of the formed adhesive interface (Rodrigues et al. 2018). Two studies compare different doses of irradiation (Biscaro et al. 2009; da Cunha et al. 2016). Biscaro et al. 2009 assessed 5 Gy, 35Gy and 70 Gy. The authors showed that adhesion resistance is dose-dependent, since the higher dosage (70 Gy) had the greatest differences on data. However, when assessing 20, 40 and 70 Gy, Cunha et al. 2016 did not observe a significant difference between irradiated and non-irradiated teeth. Both studies evaluated single dose radiotherapy. This is another important factor, since most *in vitro* studies (Bulucu et al. 2006; Freitas Soares et al. 2016; Naves et al. 2012; Rodrigues et al. 2018; Santin et al. 2018; Yadav and Yadav 2013) used a fractional dose, reproducing better what happens clinically.

Regarding the best time to perform the restorative procedure, there is a consensus in the literature. Studies show that radiotherapy significantly reduces the adhesion strength between the adhesive system and the dental tissues when restoration succeeds in radiation (Bulucu et al. 2006; Freitas Soares et al. 2016; Naves et al. 2012; Rodrigues et al. 2018; Yadav and Yadav 2013). These results may establish that the clinical restorative protocol should be performed prior to the irradiation protocol, guaranteeing better adhesive properties and consequent longevity to the restorations (Naves et al. 2012; Rodrigues et al. 2018). Of course, this will depend on many factors, from the time the patient can wait from the diagnosis of cancer to the beginning of the radiotherapy treatment. But, whenever possible, the ideal is to restore carious and non-carious lesions, as well as exchange unsatisfactory restorations, before initiation of radiotherapy (Devi and Singh 2014; Hong et al. 2010; Jansma et al. 1992; Jawad et al. 2015; Meurman and Gronroos 2010; Morais et al. 2016). This fact is justified by the alterations in enamel and dentin after radiotherapy (Table 1). Adhesion in an altered structure (after radiotherapy) prevents the formation of an optimal hybrid layer, compromising the efficiency of the adhesive restorative procedure (Freitas Soares et al. 2016; Naves et al. 2012; Rodrigues et al. 2018; Santin et al. 2015; Yadav and Yadav 2013).

Associated to changes in dental substrates, the type of adhesive system is also an important consideration. The studies about irradiated teeth show that, in general, the two step etch-and-rinse and self-etch adhesive system presented the best results. But there is still no consensus on which would be the most appropriate and few studies have compared different types of adhesive systems. Gernhardt et al. 2001; Galetti et al. 2013 and Da Cunha et al. 2016 did not detect significant differences for adhesion to irradiated dentin. However, the type of adhesive system was significant and that of three-step etch-and-rinse had the highest bond strength results (Gernhardt et al. 2001). Unlike da Cunha 2016 et al. was found that when studying two step etch-and-rinse and universal adhesive, there were high bond strength for the universal adhesive in dentin, before and after irradiation.

Three studies showed that radiotherapy and the adhesive system were significant for the results (Bernard et al. 2015; Biscaro et al. 2009; Bulucu et al. 2006). The results of Bulucu et al. 2006, show significant difference only when the two-step etch and-rinse was used, with a decrease in dentin bond strength values after radiotherapy. The two step etch-and-rinse adhesive system has shown higher bond strength values than the one and two-step self-etch comparison (Biscaro et al. 2009). The authors justify these results probably with the acid-etching step of this system, which may increase the irregularities for retention on the dentin surface (Biscaro et al. 2009). However, when comparing two-steps/self-etch adhesive system with three-steps/etch-and-rinse, the firs was more effective in irradiate dentin (Bernard et al. 2015).

The results presented show the absence of standardization and difficulty in establishing a restorative protocol for irradiated patients. The adhesion in healthy enamel and dentin is a very studied and predictable procedure, however, the results shown by the articles of this review show that there is still much to understand about adhesion to irradiated dental substrates. These results showed yet, that it is important to evaluate the performance of the acid conditioning in the irradiated enamel and dentin and the form of interaction of the acid primer of self-etching adhesive systems, to understand which adhesive system would behave better. Considering that the chemical composition of these tissues is altered, does the mechanism of formation of the hybrid layer occur in the same way? Rodrigues et al. 2018, when evaluating a three step etch-and-rinse adhesive system and irradiated dentin, showed the formation of adhesive interface with permeability characteristic due to porosity of the surface and thick primer layer. New studies evaluating the adhesive interface formed by different adhesive systems become important in the search for a better understanding of adhesion to irradiated enamel and dentin.

The study of adhesion in teeth of patients with head and neck cancer is not restricted to restorative dentistry. Two studies evaluated adhesion of orthodontic brackets to the enamel after radiotherapy. Fragments of enamel subjected to gamma irradiation present less adhesion resistance to metallic and ceramic brackets, the latter being the most damaged (Madrid Troconis et al. 2017). The resin-modified glass ionomer cement and the composite resin would be the material of choice for the bonding of the brackets in patients with radiotherapy history in the head and neck. The use of resin-modified glass ionomer cement could lead also to some fluoride ions release around the bracket, aiding the enamel remineralization (Santin et al. 2018). This is another concern that shows the importance of adhesion studies on irradiated enamel.

The management of patients after radiotherapy treatment in the region of head and neck is a great challenge for the dentist. Currently, with the advancement of the medicine, it is common for patients to be cured of cancer; however, the side effects present in the oral cavity can compromise quality of life. The indirect damage of radiotherapy, such as hyposalivation, mucositis, hygiene difficulties and prevalence of a cariogenic diet, contribute to the establishment and progression of radiation-related caries. These lesions, which are quite destructive, and the difficulty in treating them with longevity, show the importance of this line of research for a better clinical experience with this group of patients.

Well-founded studies confirm the involvement of biomechanical and micromorphological alterations in irradiated dental structures. However, when it comes to adhesion, there is a heterogeneity of results. This work sought to condense the most relevant information on the subject in order to guide and emphasize the importance of future work that facing the establishing of protocols for the dental care of irradiated patients on head and neck region.

Conclusion

From the literature review conducted, it can be concluded that radiotherapy alters the mechanical properties and chemical composition of the enamel, dentin and dentin enamel junction. Such changes make teeth more susceptible to radiation-related caries. Adhesion to enamel and dentin is compromised when adhesive restorative procedures are performed after radiotherapy. There is still no consensus as to which adhesive system has the best results on irradiated enamel and dentin.

References

Andrews N, Griffiths C (2001) Dental complications of head and neck radiotherapy: Part 2 Aust Dent J 46:174-182

Baker DG (1982) The radiobiological basis for tissue reactions in the oral cavity following therapeutic x-irradiation. A review Arch Otolaryngol 108:21-24

Beech N, Robinson S, Porceddu S, Batstone M (2014) Dental management of patients irradiated for head and neck cancer Aust Dent J 59:20-28 doi:10.1111/adj.12134

Bernard C, Villat C, Abouelleil H, Gustin MP, Grosgogeat B (2015) Tensile Bond Strengths of Two Adhesives on Irradiated and Nonirradiated Human Dentin Biomed Res Int 2015:798972 doi:10.1155/2015/798972

Biscaro SL, Moraes RR, Correr AB, Almeida SM, Boscolo FN, Soares CJ, Correr-Sobrinho L (2009) Effect of X-ray radiation dose on the bond strength of different adhesive systems to dentin J Adhes Dent 11:355-360

Bourd-Boittin K, Fridman R, Fanchon S, Septier D, Goldberg M, Menashi S (2005) Matrix metalloproteinase inhibition impairs the processing, formation and mineralization of dental tissues during mouse molar development Exp Cell Res 304:493-505 doi:10.1016/j.yexcr.2004.11.024

Braga S, Oliveira L, Rodrigues RB, Bicalho AA, Novais VR, Armstrong S, Soares CJ (2018) The Effects of Cavity Preparation and Composite Resin on Bond Strength and Stress Distribution Using the Microtensile Bond Test Oper Dent 43:81-89 doi:10.2341/16-338-L

Bulucu B, Yesilyurt C, Cakir S, Meydan AD (2006). Influence of radiation on bond strength. J Adhes Dent. 2006 Aug;8(4):217-21.

Campi LB, Lopes FC, Soares LES, de Queiroz AM, de Oliveira HF, Saquy PC, de Sousa-Neto MD (2019) Effect of radiotherapy on the chemical composition of root dentin Head Neck 41:162-169 doi:10.1002/hed.25493

Cui FZ, Ge J (2007) New observations of the hierarchical structure of human enamel, from nanoscale to microscale J Tissue Eng Regen Med 1:185-191 doi:10.1002/term.21

da Cunha SR, Ramos PA, Haddad CM, da Silva JL, Fregnani ER, Aranha AC (2016) Effects of Different Radiation Doses on the Bond Strengths of Two Different Adhesive Systems to Enamel and Dentin J Adhes Dent 18:151-156 doi:10.3290/j.jad.a35841

de Barros da Cunha SR, Fonseca FP, Ramos P, Haddad CMK, Fregnani ER, Aranha ACC (2017) Effects of different radiation doses on the microhardness, superficial morphology, and mineral components of human enamel Arch Oral Biol 80:130-135 doi:10.1016/j.archoralbio.2017.04.007

De Felice F, Tombolini V (2018) Delineation of organs at risk in the head and neck region Oral Oncol 87:197-198 doi:10.1016/j.oraloncology.2018.10.037

de Miranda RR, Silva ACA, Dantas NO, Soares CJ, Novais VR (2018) Chemical analysis of in vivo-irradiated dentine of head and neck cancer patients by ATR-FTIR and Raman spectroscopy Clinical oral investigations doi:10.1007/s00784-018-2758-6

de Sa Ferreira EM, Soares LE, Antunes HS, Uemura ST, da Silva Barbosa P, Salmon HA, Jr., de Sant'Anna GR (2016) Effect of therapeutic doses of radiotherapy on the organic and inorganic contents of the deciduous enamel: an in vitro study Clinical oral investigations 20:1953-1961 doi:10.1007/s00784-015-1686-y

de Siqueira Mellara T, Palma-Dibb RG, de Oliveira HF, Garcia Paula-Silva FW,Nelson-Filho P, da Silva RA, da Silva LA, de Queiroz AM. (2014) The effect of radiation therapy on the mechanical and morphological properties of the enamel and dentin of deciduous teeth--an in vitro study Radiation oncology 9:30 doi:10.1186/1748-717X-9-30

Deng J, Jackson L, Epstein JB, Migliorati CA, Murphy BA (2015) Dental demineralization and caries in patients with head and neck cancer Oral Oncol 51:824-831 doi:10.1016/j.oraloncology.2015.06.009

Devi S, Singh N (2014) Dental care during and after radiotherapy in head and neck cancer Natl J Maxillofac Surg 5:117-125 doi:10.4103/0975-5950.154812

Dreizen S, Brown LR, Daly TE, Drane JB (1977a) Prevention of xerostomia-related dental caries in irradiated cancer patients J Dent Res 56:99-104 doi:10.1177/00220345770560022101

Dreizen S, Daly TE, Drane JB, Brown LR (1977b) Oral complications of cancer radiotherapy Postgrad Med 61:85-92

Epstein JB, Chin EA, Jacobson JJ, Rishiraj B, Le N (1998) The relationships among fluoride, cariogenic oral flora, and salivary flow rate during radiation therapy Oral Surg Oral Med Oral Pathol Oral Radiol Endod 86:286-292

Franzel W, Gerlach R, Hein HJ, Schaller HG (2006) Effect of tumor therapeutic irradiation on the mechanical properties of teeth tissue Z Med Phys 16:148-154

Freitas Soares E, Zago Naves L, Bertolazzo Correr A, Costa AR, Consani S, Soares CJ, Garcia-Godoy F, Correr-Sobrinho L. (2016) Effect of radiotherapy, adhesive systems and doxycycline on the bond strength of the dentin-composite interface Am J Dent 29:352-356

Galetti R, Santos-Silva AR, Antunes AN, Alves Fde A, Lopes MA, de Goes MF (2014) Radiotherapy does not impair dentin adhesive properties in head and neck cancer patients Clinical oral investigations 18:1771-1778 doi:10.1007/s00784-013-1155-4

Gernhardt CR, Kielbassa AM, Hahn P, Schaller HG (2001) Tensile bond strengths of four different dentin adhesives on irradiated and non-irradiated human dentin in vitro J Oral Rehabil 28:814-820

Goldberg M, Kulkarni AB, Young M, Boskey A (2011) Dentin: structure, composition and mineralization Front Biosci (Elite Ed) 3:711-735

Goncalves LM, Palma-Dibb RG, Paula-Silva FW, Oliveira HF, Nelson-Filho P, Silva LA, Queiroz AM (2014) Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth J Dent 42:986-992 doi:10.1016/j.jdent.2014.05.011

Gupta N Pal M, Rawat S, Grewal MS, Garg H, Chauhan D, Ahlawat P, Tandon S,Khurana R, Pahuja AK, Mayank M, Devnani B (2015) Radiation-induced dental caries, prevention and treatment - A systematic review Natl J Maxillofac Surg 6:160-166 doi:10.4103/0975-5950.183870

Hay KD, Thomson WM (2002) A clinical trial of the anticaries efficacy of casein derivatives complexed with calcium phosphate in patients with salivary gland dysfunction Oral Surg Oral Med Oral Pathol Oral Radiol Endod 93:271-275

Hong CH et al. (2010) A systematic review of dental disease in patients undergoing cancer therapy Support Care Cancer 18:1007-1021 doi:10.1007/s00520-010-0873-2

Imbeni V, Kruzic JJ, Marshall GW, Marshall SJ, Ritchie RO (2005) The dentin-enamel junction and the fracture of human teeth Nat Mater 4:229-232 doi:10.1038/nmat1323

Jansma J, Vissink A, Spijkervet FK, Roodenburg JL, Panders AK, Vermey A, Szabó BG, Gravenmade EJ. (1992) Protocol for the prevention and treatment of oral sequelae resulting from head and neck radiation therapy Cancer 70:2171-2180

Jawad H, Hodson NA, Nixon PJ (2015) A review of dental treatment of head and neck cancer patients, before, during and after radiotherapy: part 1 Br Dent J 218:65-68 doi:10.1038/sj.bdj.2015.28

Jongebloed WL, Gravenmade EJ, Retief DH (1988) Radiation caries. A review and SEM study Am J Dent 1:139-146

Kielbassa AM, Beetz I, Schendera A, Hellwig E (1997) Irradiation effects on microhardness of fluoridated and non-fluoridated bovine dentin European journal of oral sciences 105:444-447

Kielbassa AM, Hinkelbein W, Hellwig E, Meyer-Luckel H (2006) Radiation-related damage to dentition Lancet Oncol 7:326-335 doi:10.1016/S1470-2045(06)70658-1

Kielbassa AM, Munz I, Bruggmoser G, Schulte-Monting J (2002) Effect of demineralization and remineralization on microhardness of irradiated dentin The Journal of clinical dentistry 13:104-110

Liang X, Zhang JY, Cheng IK, Li JY (2016) Effect of high energy X-ray irradiation on the nano-mechanical properties of human enamel and dentine Brazilian oral research 30 doi:10.1590/1807-3107BOR-2016.vol30.0009

Lieshout HF, Bots CP (2014) The effect of radiotherapy on dental hard tissue--a systematic review Clinical oral investigations 18:17-24 doi:10.1007/s00784-013-1034-z

Lopes CCA, Soares CJ, Lara VC, Arana-Chavez VE, Soares PB, Novais VR (2018) Effect of fluoride application during radiotherapy on enamel demineralization J Appl Oral Sci 27:e20180044 doi:10.1590/1678-7757-2018-0044

Lu H, Zhao Q, Guo J, Zeng B, Yu X, Yu D, Zhao W (2019) Direct radiation-induced effects on dental hard tissue Radiation oncology 14:5 doi:10.1186/s13014-019-1208-1

Marur S, Forastiere AA (2016) Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment Mayo Clin Proc 91:386-396 doi:10.1016/j.mayocp.2015.12.017

McGuire JD, Gorski JP, Dusevich V, Wang Y, Walker MP. Type IV collagen is a novel DEJ biomarker that is reduced by radiotherapy. J Dent Res. 2014 Oct;93(10):1028-34. doi: 10.1177/0022034514548221.

Meurman JH, Gronroos L (2010) Oral and dental health care of oral cancer patients: hyposalivation, caries and infections Oral Oncol 46:464-467 doi:10.1016/j.oraloncology.2010.02.025

Miguez PA, Pereira PN, Atsawasuwan P, Yamauchi M (2004) Collagen cross-linking and ultimate tensile strength in dentin J Dent Res 83:807-810 doi:10.1177/154405910408301014

Mjor IA (1972) Human coronal dentine: structure and reactions Oral Surg Oral Med Oral Pathol 33:810-823

Morais MO, Elias MR, Leles CR, Dourado Pinezi JC, Mendonca EF (2016) The effect of preventive oral care on treatment outcomes of a cohort of oral cancer patients Support Care Cancer 24:1663-1670 doi:10.1007/s00520-015-2956-6

Naves LZ, Novais VR, Armstrong SR, Correr-Sobrinho L, Soares CJ (2012) Effect of gamma radiation on bonding to human enamel and dentin Support Care Cancer 20:2873-2878 doi:10.1007/s00520-012-1414-y

Novais VR, Soares PB, Guimaraes CM, Schliebe LR, Braga SS, Soares CJ (2016) Effect of Gamma Radiation and Endodontic Treatment on Mechanical Properties of Human and Bovine Root Dentin Braz Dent J 27:670-674 doi:10.1590/0103-6440201601267

Papas A, Russell D, Singh M, Kent R, Triol C, Winston A (2008) Caries clinical trial of a remineralising toothpaste in radiation patients Gerodontology 25:76-88 doi:10.1111/j.1741-2358.2007.00199.x

Pioch T, Golfels D, Staehle HJ (1992) An experimental study of the stability of irradiated teeth in the region of the dentinoenamel junction Endod Dent Traumatol 8:241-244

Qing P, Huang S, Gao S, Qian L, Yu H (2015) Effect of gamma irradiation on the wear behaviour of human tooth enamel Scientific reports 5:11568 doi:10.1038/srep11568

Qing P, Huang S, Gao S, Qian L, Yu H (2016) Effect of gamma irradiation on the wear behavior of human tooth dentin Clinical oral investigations 20:2379-2386 doi:10.1007/s00784-016-1731-5

Queiroz AM, Bonilla CMC, Palma-Dibb RG, Oliveira HF, Nelson-Filho P, Silva LAB, Lucisano MP, Paula-Silva FWG (2018) Radiotherapy Activates and Protease Inhibitors Inactivate Matrix Metalloproteinases in the Dentinoenamel Junction of Permanent Teeth Caries Res 53:253-259 doi:10.1159/000492081

Ray-Chaudhuri A, Shah K, Porter RJ (2013) The oral management of patients who have received radiotherapy to the head and neck region Br Dent J 214:387-393 doi:10.1038/sj.bdj.2013.380

Reed R, Xu C, Liu Y, Gorski JP, Wang Y, Walker MP (2015) Radiotherapy effect on nano-mechanical properties and chemical composition of enamel and dentine Arch Oral Biol 60:690-697 doi:10.1016/j.archoralbio.2015.02.020

Rodrigues RB, Soares CJ, Junior PCS, Lara VC, Arana-Chavez VE, Novais VR (2018) Influence of radiotherapy on the dentin properties and bond strength Clinical oral investigations 22:875-883 doi:10.1007/s00784-017-2165-4

Sahlberg C, Reponen P, Tryggvason K, Thesleff I (1999) Timp-1, -2 and -3 show coexpression with gelatinases A and B during mouse tooth morphogenesis European journal of oral sciences 107:121-130

Santin GC, Palma-Dibb RG, Romano FL, de Oliveira HF, Nelson Filho P, de Queiroz AM (2015) Physical and adhesive properties of dental enamel after radiotherapy and bonding of metal and ceramic brackets Am J Orthod Dentofacial Orthop 148:283-292 doi:10.1016/j.ajodo.2015.03.025

Santin GC, Queiroz AM, Palma-Dibb RG, Oliveira HF, Nelson Filho P, Romano FL (2018) Glass Ionomer Cements can be used for Bonding Orthodontic Brackets After Cancer Radiation Treatment? Braz Dent J 29:128-132 doi:10.1590/0103-6440201801436

Seyedmahmoud R, Wang Y, Thiagarajan G, Gorski JP, Reed Edwards R, McGuire JD, Walker MP (2018) Oral cancer radiotherapy affects enamel microhardness and associated indentation pattern morphology Clinical oral investigations 22:1795-1803 doi:10.1007/s00784-017-2275-z

Silva AR, Alves FA, Antunes A, Goes MF, Lopes MA (2009) Patterns of demineralization and dentin reactions in radiation-related caries Caries Res 43:43-49 doi:10.1159/000192799

Soares CJ, Castro CG, Neiva NA, Soares PV, Santos-Filho PC, Naves LZ, Pereira PN (2010) Effect of gamma irradiation on ultimate tensile strength of enamel and dentin J Dent Res 89:159-164 doi:10.1177/0022034509351251

Soares CJ, Neiva NA, Soares PB, Dechichi P, Novais VR, Naves LZ, Marques MR (2011) Effects of chlorhexidine and fluoride on irradiated enamel and dentin J Dent Res 90:659-664 doi:10.1177/0022034511398272

Strup-Perrot C, Vozenin-Brotons MC, Vandamme M, Benderitter M, Mathe D (2006) Expression and activation of MMP -2, -3, -9, -14 are induced in rat colon after abdominal X-irradiation Scand J Gastroenterol 41:60-70 doi:10.1080/00365520510023963

Thiagarajan G et al. (2017) Stress analysis of irradiated human tooth enamel using finite element methods Comput Methods Biomech Biomed Engin 20:1533-1542 doi:10.1080/10255842.2017.1383401

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012 CA Cancer J Clin 65:87-108 doi:10.3322/caac.21262

Tschiesner U (2012) Preservation of organ function in head and neck cancer GMS Curr Top Otorhinolaryngol Head Neck Surg 11:Doc07 doi:10.3205/cto000089

Urabe I, Nakajima S, Sano H, Tagami J (2000) Physical properties of the dentin-enamel junction region Am J Dent 13:129-135

Velo M et al. (2018) Radiotherapy alters the composition, structural and mechanical properties of root dentin in vitro Clinical oral investigations 22:2871-2878 doi:10.1007/s00784-018-2373-6

Vissink A, Jansma J, Spijkervet FK, Burlage FR, Coppes RP (2003) Oral sequelae of head and neck radiotherapy Crit Rev Oral Biol Med 14:199-212

Walker MP, Wichman B, Cheng AL, Coster J, Williams KB (2011) Impact of Radiotherapy Dose on Dentition Breakdown in Head and Neck Cancer Patients Pract Radiat Oncol 1:142-148 doi:10.1016/j.prro.2011.03.003

Yadav S, Yadav H (2013) Ionizing irradiation affects the microtensile resin dentin bond strength under simulated clinical conditions J Conserv Dent 16:148-151 doi:10.4103/0972-0707.108198

3.2 Capítulo 2

Effect of radiotherapy on the bond strength of composite resin to human enamel

Artigo submetido para o periódico *Radiation and Environmental Biophysics*

Effect of radiotherapy on the bond strength of composite resin to human enamel

Abstract

The purpose of this study was evaluating the microshear bond strength (μSBS) of composite resin to human enamel performed before or after radiotherapy (RT). Forty-five humans third molars were randomly divided into three groups (n=15): CT- control/no irradiated; IA- irradiated after restorative procedure and IB- irradiated before restorative procedure. The experimental groups were exposed to high energy X-ray doses, applied 2 Gy daily, 5 days per week, for 7 weeks. After surface treatment and hybridization, two tygon tubing were placed on the buccal surface of the enamel and filled with nanohybrid composite resin. The μSBS was assessed by microshear bond strength test (0.5 mm/min). After the mechanical test, the specimens were submitted to scanning electron microscopy (SEM) to evaluate the failure mode. Analysis of the bonding interface was made by Confocal Microscopy. Data were submitted to one-way ANOVA followed by Tukey's HSD test ($p<0.05$). The failure mode was analyzed descriptively. CT and IA groups showed similar bond strength values ($p=0.716$), while the IB group presented the lowest bond strength values ($p\leq0.001$). For the failure mode analysis, the CT and IB groups showed predominance of adhesive failures, while the IA group presented predominance of cohesive failures in enamel. It was found that specimens irradiated before the restorative procedures showed lower bond strength values. Greater prevalence of cohesive failures in enamel were verified for the group restored before the radiotherapy protocol. Confocal microscopy revealed the formation a heterogeneous adhesive interface for IB group.

Key Words: Adhesion, Dental enamel, Head and Neck Neoplasms; Radiotherapy.

INTRODUCTION

The incidence of head and neck cancer have increased worldwide (Ferlay et al. 2015). Radiotherapy is an important stage for the treatment of this disease (Kielbassa et al. 2006). It may be recommended as the primary therapy, as adjuvant to surgery and chemotherapy, or a palliative treatment for inoperable cases and the final stages of the disease (Buglione et al. 2016). Despite being a highly effective non-invasive treatment for tumor control, radiotherapy causes many adverse reactions that significantly affect the patient´s quality of life (Kielbassa et al. 2006; Buglione et al. 2016; Deng et al. 2015; Vissink et al. 2003).

When the radiation field involves the oral cavity and teeth, side effects including reduction in salivary flow, changes in the saliva composition with a reduction in the protective properties and salivary pH can occur (Deng et al. 2015; Lieshout et al. 2014). Besides, quantitative and qualitative changes in bacterial flora and poor hygiene makes the oral environment highly susceptible to the caries development (Kielbassa et al. 2006; Deng et al. 2015; Jawad et al. 2015; Lieshout et al. 2014). Thus, radiation caries is one of the main side-effects of head and neck radiotherapy and is very harmful to dental structure (Kielbassa et al. 2006; Vissink et al. 2003; Jawad et al. 2015; Lieshout et al. 2014; Deng et al. 2015; Jawad et al. 2015; Lieshout et al. 2014). These lesions present rapid onset and progression, with a great potential for destroying the teeth. Its evolution can result in amputation of the dental crown, which can progress to a complete loss of the tooth in a short period of time (Kielbassa et al. 2006; Deng et al. 2015; Vissink et al. 2003; Jawad et al. 2015; Lieshout et al. 2014).

Associated with modifications in the buccal environment, dental hard tissues are changed by radiotherapy. A considerable number of *in vitro* studies have demonstrated that high doses of ionizing radiation may impair the properties of enamel and dentin and the stability of dentin-enamel junction (Kielbassa et al. 2006; Vissink et al. 2003; Lieshout et al. 2014; Novais et al. 2016; Gonçalves et al. 2014; Reed et al. 2015; Santin et al. 2015; Qing et al. 2015; Rodrigues et al. 2018; Sevedmahmound et al. 2018; Abdalla et al. 2018). In enamel, a interprismatic disorganization structure, demineralization and decreased microhardness, especially in the cervical region, favors the rapid development of caries (Gonçalves et al. 2014; Sevedmahmound et al. 2018; Abdalla et al. 2018). This occurs because of the decarboxylation caused by radiation (Hubner et al. 2005) and additionally there is a reduction in the interaction between organic components and hydroxyapatite crystals of both enamel and dentin (Lieshout et al. 2014; Rodrigues et al. 2018; Sevedmahmound et al. 2018).

Such alterations, besides making the tissue fragile and more susceptible to caries development, difficult the restorative process with adhesive materials (Rodrigues et al. 2018; Naves et al. 2012; Gernhardt et al. 2001; da Cunha et al. 2016). The literature is still controversial regarding the mechanisms of adhesion in irradiated enamel and dentin. Distinct protocols used in some studies show different results in relation to the bond strength of adhesive restorative materials to irradiated enamel and dentin (Rodrigues et al. 2018; Naves et al. 2012; Gernhardt et al. 2001; da Cunha et al. 2016). The difficult in the restorative treatment highlight the importance of studies on the mechanism of adhesion in irradiated teeth. It is known that radiation caries progresses are very fast and for that reason the majority of adhesion area is realized in dentin. However, the restoration margins will be in enamel and/or cement, depending on the extent of the lesion. The presence of the enamel at the restoration margins plays an important role in the protection of the resin–dentin interface against degradation (Gamborgi et al., 2007). Thus, ensuring efficient adhesion to the enamel is a key factor for the effectiveness and efficiency of a restoration (Gamborgi et al., 2007). In addition, it is common the involvement of radiations caries in cusps tip and incisal of anterior teeth (Kielbassa et al. 2006). And both regions have a greater thickness of enamel. In this way the study of the adhesion in irradiated enamel is of great importance. The null-hypothesis of the present study is that the restoration placement moment (before or after) radiotherapy would not influence the bond strength of a composite resin material to human enamel.

MATERIAL AND METHODS

Forty-five non-carious humans third molars were collected, cleaned and stored in distilled water at 4°C up to six months after extraction. This study was approved by the Ethical Committee in Research (Protocol #1452041).

The selected teeth were randomly divided into three groups (n=15): CT- control/no irradiated; IB- irradiated before restorative procedure; and IA- irradiated after restorative procedure. The radiotherapy protocol for the experimental groups consisted of total of 70 Gy, with 2 Gy exposure applied 5 days per week, during 7 weeks using a linear accelerator (Clinac 600C Varian® - Palo Alto, CA, USA, Beam 6 MV). The specimens were stored in distilled water changed weekly during the irradiation process (Rodrigues et al. 2018). The placement of the beam and the radiation dose was calculated according to a mean usually applied on patients with head and neck cancers in the radiotherapy division from the Hospital of the Federal University of Triangulo Mineiro (UFTM, Uberaba, Minas Gerais, Brazil).

Specimen preparation

The crowns were separated from the roots using a double-faced diamond disc (Extec, Enfield, CT, USA) mounted in a precision cutter (Isomet 1000, Buehler, Lake Bluff, IL, USA). The crowns were included in polystyrene resin cylinders (Aerojet, Santo Amaro, SP, Brazil) (Fig. 1A) and had their buccal enamel surfaces polished using No. 600 and No. 1200 grit silicon carbide abrasive papers under constant irrigation, for 20 seconds each, in a polishing machine (Arotec Ind., Cotia, SP, Brazil). Specimens were placed in an ultrasonic bath (USC1400, Unique, Indaiatuba, SP, Brazil) for 15 minutes at the end of the polishing procedure to remove debris and then analyzed under optical microscopy (Mitutoyo, Suzano, SP, Brazil) to assure the buccal surfaces were still located in enamel.

Then, the restorative procedure was performed according to the parameters of the experimental groups. The following protocol was used to restore the groups: prophylaxis with a rubber cup and pumice (Asfer, Sao Caetano do Sul, SP, Brazil) (Fig. 1B) , 37% phosphoric-acid etching (FGM, Joinville, SC, Brazil) for 30 s on enamel (Fig. 1C), air-water washing spray for 30 s and drying with absorbent paper, application of the primer of the etch-and-rinse adhesive system (Scotchbond Multi-Purpose, 3M-ESPE, St. Paul, MN, USA), followed by gently air-dry for 5 s, application of the bond component (Scotchbond

Multi-Purpose, 3M-ESPE) (Fig. 1D), and light-curing for 20 s with a LED unit (Bluephase N, Ivoclar Vivadent, Schaan, Liechtenstein - 1200 mW/cm²). After that, two tygon bore tubing (0.75 mm diameter x 1 mm high) were placed on the prepared enamel surface (Fig. 1F). One increment of a nanofilled composite resin (Filtek Z350 XT, A2 shade, 3M-ESPE) was inserted into the tygon tubing (Fig. 1G) and photoactivated for 20 s by a LED unit (Bluephase N - 1200 mW/cm²) (Fig. 1H). After 24 h, the tygon tubing were removed with scalpel blades (Figs. 1I and 1J) and the specimens were stored in distilled water.

Microshear Bond Strength Test (μSBS)

After the storage, each sample was fixed in a microshear device coupled to a mechanical testing machine (Microtensile OM100, Odeme Dental Research, Luzerna, SC, Brazil). A stainless steel wire (0.2 mm diameter, Morelli, Sorocaba, SP, Brazil) was placed around each resin cylinder parallel to loading direction. The microshear force was applied at a crosshead speed of 0.7 mm/min until failure occurred. The microshear bond strength results were calculated in MPa using the following formula: R = Rupture Force (*Kgf*) x 9.8 / Area (mm^2) where R is the bond strength in MPa.

Scanning electron microscopy (SEM)

After the microshear test, the specimens were analyzed under scanning electron microscopy for defining the failure pattern. For this purpose, the specimens were cleaned in ultrasonic bath (USC1400) with distilled water for three cycles of 10 min. Then, specimens were dried at 37°C for 24 h and fixed on aluminum stubs using a conductive carbon tape, with the buccal surfaces positioned face up. A thin gold-palladium film (10 μm thickness) was deposited on the surface of the specimens (Bal-Tec SCD-050 sputtering system, Leica Microsystems, Wetzlar, Germany). After the gold-sputtering, the enamel surfaces were observed under a scanning electron microscope (EVO MA10, Zeiss, Oberkochen, Germany), operated at 15 kV, in the secondary electrons mode.

The classification of failure modes was made according to the Table 1.

Confocal Laser Scanning Microscopy (CLSM)

Following the same adhesive protocols described above, one specimen per group were prepared for observation under CLSM (Rodrigues et al. 2018). The colorant Sodium-salt Fluorescein (Sigma - St Louis, MO, EUA) was incorporated into the primer and the Rhodamine B (Sigma, EUA) into the bond of adhesive system. The teeth were then cut longitudinally into two halves and both surfaces were polished for one minute with SiC papers (Grit 800/1200/1500) and were examined under a CLSM (LSM 510 Meta, Zeiss, Oberkochen, Germany). Fluorescein was excited at 488-nm excitation line of Argon ion laser and Rhodamine B was excited at 543-nm excitation line of Helium ion laser. CLSM images were obtained from 3 μm slices throughout the depth of the sample. Each resin–enamel interface was completely investigated and then five optical images were randomly captured. Micrographs representing the most common features observed along the bonded interfaces were captured and recorded.

STATISTICAL ANALYSIS

Microshear bong strength data were tested for normal distribution (Shapiro-Wilk, $p>0.05$) and equality of variances (Levene's test, $p>0.05$). The μSBS values were submitted to one-way ANOVA followed by Tukey's test. All tests employed at $\alpha=0.05$ level of significance and were made by Sigma Plot statistical package (12.0, Systat Software, Inc., San Jose, CA, USA). Descriptive analysis was used to evaluated confocal images and classify the failure mode.

RESULTS

Microshear Bond Strength (μSBS)

The mean bond strength values and standard deviation for the experimental groups are shown in Table 2. One-way ANOVA revealed significant differences among the groups ($p<0.001$). The CT and IA groups were similar and presented the highest bond strength values ($p=0.716$). On the other hand, the IB group showed the lowest bond strength values, being statistically different from the other groups ($p\leq0.001$).

Failure mode

The results of SEM analysis for the failure mode are summarized in Figs. 2 and 3 and table 3. The CT group exhibited 50% of adhesive failures, 25% of mixed failures, 14.28% of cohesive failures in enamel and 10.72% of cohesive failures in composite resin. The IA group presented the highest number of cohesive failures in enamel (39.29%), followed by 35.71% of adhesive failures, 21.43% of mixed failures and 3.57% of cohesive failures in composite resin. The IB group showed 46.43% of adhesive failures, 32.15% mixed of failures, 14.28% of cohesive failures in enamel and 7.14% of cohesive failures in composite resin.

CLSM

Figure 4 shows the adhesive interface pattern of each of the groups. One can observe the interaction of the primer and adhesive with the enamel (E) and the adhesive layer (AL) formed in the CT and IA groups. For both, a homogeneous adhesive interface is observed, with mechanical bonding between adhesive system and enamel. In the IB group, a heterogeneous adhesive interface is observed, as a clear division between primer and adhesive. It also observed areas of failure with a pattern of irregular mechanical imbrication between adhesive system and enamel.

DISCUSSION

The null hypothesis tested in this study was rejected, since the group irradiated before the restorative procedure (IB) presented lower bond strength values between composite resin and enamel. This result shows that the moment of restoration placement (before or after the radiotherapy) was important for the adhesion strength between composite resin and enamel.

To understand the adhesion process in irradiated enamel is necessary to consider the composition and microstructure of this tissue. Enamel is a highly mineralized tissue consisting predominantly of carbonated hydroxyapatite (96%) with 3% water and a very small amount of organic matrix (~1%wt.) (Fincham et al. 1999). Composite materials, usually present excellent bond strength to enamel due to the micromechanical interlocking that is created by acid etching, creating micropososities through a selective dissolution of the enamel prisms followed by the use of an adhesive that penetrates these porosities (Buonocore et al. 1968). This mechanism is explained by the increase of enamel surface energy that happens through two stages: first, there is removal of mineral and other deposits together with a layer of enamel; and second, there is an increase in the surface porosities creating microporosities, which provide a better contact surface, where the adhesive system will infiltrate previously to photoactivation (Buonocore et al. 1968). However, the results showed that enamel irradiated before the restorative procedure presented lower bond strength values compared both to non-irradiated enamel and enamel irradiated after restorative procedure.

The literature leaves no doubt about the changes that head and neck radiotherapy causes in the structure and mechanical properties of enamel (Soares et al. 2010; Lieshout et al. 2014; Gonçalves et al. 2014; Reed et al. 2015; Santin et al. 2015; Qing et al. 2015; Seyedmahmoud et al. 2018; Abdalla et al. 2018). Radiotherapy acts in the neoplasic tissue through the formation of free radicals of hydrogen and hydrogen peroxide (Pioch et al. 1992). These free radicals, in the presence of water, are able to act as a strong oxidant that may cause denaturation of the molecular structure (Pioch et al. 1992). In the enamel, the very little amount of water is located in the interprismatic region. Damage from free radical and reactive oxygen species accumulation, may react with and damage organic components (Xu et al. 2012). The organic matrix is present in dental enamel at very low concentrations (1%), but plays a fundamental role for maintaining the stability of this tissue and changes in this content can affect its mechanical properties (Baldassarri et al. 2008). Composed of small peptides and amino acids that are distributed throughout mature tissue, the organic matrix provides the model for enamel mineralization and plays an important role in the control of ionic diffusion in this tissue (Liu et al. 2007). In this way, it prevents, facilitates or manages enamel demineralization (Liu et al. 2007). The compromise of the organic matrix leaves the enamel more friable (Santin et al. 2015). Considering that, the adhesion to dental enamel depends on its superficial demineralization, alterations caused by radiotherapy in the interprismatic region and in the organic matrix may compromise the formation of an ideal bonding interface, thus affecting the bond strength of adhesive materials to irradiated enamel.

Studies show that the results of michoshear test are reliable and provide conclusions similar to the microtensile test (Andrade et al. 2010; Beloica et al. 2010). Thus, because it is an evaluation of bond strength only in enamel, this study used the microshear mechanical test, due to the difficulty of obtaining samples for the microtensile test in enamel. The literature shows other studies that also used this test to evaluate adhesion in enamel (da Cunha et al. 2016; Nagura et al. 2018). The results (Table 2) show that the group that was irradiated before the restorative process (IB) showed the lowest bond strength values among the groups. This shows that the adhesive procedure was not efficient for establishing adequate bonding to the irradiated enamel. This happened due to the alterations caused by the high radiation dose (70 Gy) that the tooth received previous to the restorative procedure, which alter the enamel structure (Soares et al. 2010; Lieshout et al. 2014; Gonçalves et al. 2014; Reed et al. 2015; Santin et al. 2015; Qing et al. 2015; Seyedmahmoud et al. 2018; Abdalla et al. 2018). When comparing this result with the images obtained by CLSM (figure 4), it can be seen that the formation of a heterogeneous adhesive interface in IB group that justifies the result found in the microshear test. It is suggested that the alteration caused by radiotherapy, especially in the interprismatic region, compromises a correct demineralization pattern by the acid conditioning, with consequent alteration in the penetration of the primer and adhesive, which compromises the bond strength of the resin composite to the irradiated enamel. The group irradiated after the restorative procedure (IA) presented mean bond strength values similar to the non-irradiated group (CT). CLSM images corroborate with these results, since it is possible to observe a homogeneous adhesive interfacial pattern in the control group as well as in the IA group. This occurred because the adhesive procedure was performed on a healthy tissue, ensuring good bond strength results.

The results shown by the failure pattern analysis evidence alterations in enamel after radiotherapy. IA group showed the highest percentages of cohesive failures in enamel (Fig. 3 and Table 3). It becomes clear that radiotherapy was not able to compromise the adhesive interface that was already formed, but it has compromised the enamel structure, leaving it more susceptible to fracture when a shear force was applied. It is possible to associate this amount of cohesive failures to the alteration that occurs in the interprismatic region, where protein part and water of the enamel are concentrated (al-Nawas et al. 2000). This concentration of organic matrix ensures a better distribution of force between the prisms (Baldassarri et al. 2008). With its degradation by radiotherapy (al-Nawas et al. 2000), the prisms become more fragile since there is a diminution of its capacity to absorb and dissipate energy (Santin et al. 2015). In addition, the degradation of water molecules results in a dehydrated tissue which consequently becomes more susceptible to fractures (Santin et al. 2015). Already for the CT and IB the predominant failure mode was composed by a high percentage of adhesive failures. Predominance of adhesive failures guarantee reliability to the micro-shear test. Thus, when comparing the two groups, the adhesive resistance values of the IB group were significantly lower than the CT group. These results confirm the poor performance of the adhesive system in the irradiated enamel and the fragility of the adhesive interface formed between restorative material and enamel after radiotherapy.

The literature is still controversial about the influence of radiotherapy on the adhesion to dental tissues. But the constant clinical failures observed in in adhesive restorations of patients submitted to radiotherapy continue to motivate studies and awaken the professionals who work with these patients on the importance of the continuous research in this field. With the advancement of cancer studies and early diagnosis of the disease, the cure and survival rates have increased. Thus, studies seeking to understand the sequelae caused by this treatment become of great importance in the search for quality of life for these patients after cancer treatment. Knowing the radiation contributes to demineralization of tooth enamel (Abdalla et al. 2018) becomes relevant further studies on adhesion to this tissue. A study of this same group showed that radiotherapy compromises adhesion to dentin (Rodrigues et al. 2018), and the results of this paper show the same behavior for enamel adhesion, that it is more appropriate to restore all carious lesions and to change unsatisfactory restorations prior to the radiotherapy treatment protocol. Considering the rapid progression of the caries of radiation it is known that the reality of the clinicians is the realization of restorations in dentin (mainly in the cervical region), with the margins in enamel and / or cement. It is therefore important to understand the adhesive procedure of the two tissues. It is important to continue searching for information to define a better protocol for these patients. New in vivo studies that accurately replicate the environment in which restorations are made and the challenges for their maintenance will add novel information for better understanding the complex treatment of these patients.

CONCLUSION

The teeth restored after the radiotherapy protocol showed lower bond strength values among resin composite and enamel. Greater prevalence of cohesive failures in enamel were verified for the group restored before the radiotherapy protocol. Confocal microscopy revealed the formation a deficient adhesive interface for IB group.

ACKNOWLEDGEMENTS

This research was supported by the Minas Gerais State Agency for Research and Development (FAPEMIG), National Council for Scientific and Technological Development (CNPq) and Coordination of Personal Development at a Higher Level (CAPES).

The authors are indebted to the Scanning Electron Microscopy Laboratory of the School of Chemical Engineering of Federal University of Uberlandia (FEQUI-UFU) for the SEM analysis.

The authors are indebted the Radiotherapy Sector of the Federal University of Triangulo Mineiro for the irradiation of the samples.

REFERENCES

Abdalla R, Omar A, Eid K (2018) Detecting demineralization of enamel and cementum after gamma irradiation using radiographic densitometry. Radiat Environ Biophys. Aug;57(3):293-299.

al-Nawas B, Grötz KA, Rose E, Duschner H, Kann P, Wagner W (2000) Using ultrasound transmission velocity to analyse the mechanical properties of teeth after in vitro, in situ, and in vivo irradiation. Clin Oral Investig. Sep;4(3):168-72

Andrade AM, Moura SK, Reis A, Loguercio AD, Garcia EJ, Grande RH (2010) Evaluating resin-enamel bonds by microshear and microtensile bond strength tests: effects of composite resin. J Appl Oral Sci. Dec;18(6):591-8

Baldassarri M, Margolis HC, Beniash E (2008) Compositional determinants of mechanical properties of enamel. J Dent Res. Jul;87(7):645-9

Beloica M, Goracci C, Carvalho CA, Radovic I, Margvelashvili M, Vulicevic ZR, Ferrari M (2010) Microtensile vs microshear bond strength of all-in-one adhesives to unground enamel. J Adhes Dent. Dec;12(6):427-33.

Buglione M, Cavagnini R, Di Rosario F, Sottocornola L, Maddalo M, Vassalli L et al. (2016) Oral toxicity management in head and neck cancer patients treated with chemotherapy and radiation: dental pathologies and osteoradionecrosis (Part 1) literature review and consensus statement. Crit Rev Oncol Hematol. 97:131–142.

Buonocore MG, Matsui A, Gwinnett AJ (1968) Penetration of resin dental materials into enamel surfaces with reference to bonding. Arch Oral Biol 13:61-70

da Cunha SR, Ramos PA, Haddad CM, da Silva JL, Fregnani ER, Aranha AC (2016) Effects of Different Radiation Doses on the Bond Strengths of Two Different Adhesive Systems to Enamel and Dentin. J Adhes Dent 18(2):151-6.

Deng J, Jackson L, Epstein JB, Migliorati CA, Murphy BA (2015) Dental demineralization and caries in patients with head and neck cancer. Oral Oncol 51(9):824–831.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359-386.

Fincham AG, Moradian-Oldak J, Simmer JP (1999) The structural biology of the developing dental enamel matrix. J Struct Biol Jun 30;126(3):270-99.

Gernhardt CR, Kielbassa AM, Hahn P, Schaller HG (2001) Tensile bond strengths of four different dentin adhesives on irradiated and non-irradiated human dentin in vitro. J Oral Rehabil Sep;28(9):814-20.

Gonçalves LM, Palma-Dibb RG, Paula-Silva FW, Oliveira HF, Nelson-Filho P, Silva LA, Queiroz AM (2014) Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth. J Dent Aug;42(8):986-92.

Hübner W, Blume A, Pushnjakova R, Dekhtyar Y, Hein HJ (2005) The influence of X-ray radiation on the mineral/organic matrix interaction of bone tissue: an FT-IR microscopic investigation. Int J Artif Organs Jan;28(1):66-73.

Jawad H, Hodson NA, Nixon PJ (2015) A review of dental treatment of head and neck cancer patients, before, during and after radiotherapy: part 1. Br Dent J. Jan;218(2):65-8.

Kielbassa AM, Hinkelbein W, Hellwig E, Meyer-Lückel H (2006) Radiation-related damage to dentition. Lancet Oncol. 7(4):326–335.

Lieshout HF, Bots CP (2014) The effect of radiotherapy on dental hard tissue—a systematic review. Clin Oral Investig. Jan;18(1):17-24.

Liu Y, Hsu CY (2007) Laser-induced compositional changes on enamel: a FT-Raman study. J Dent. Mar;35(3):226-30.

Nagura Y, Tsujimoto A, Fischer NG, Baruth AG, Barkmeier WW, Takamizawa T, Latta MA, Miyazaki M (2018) Effect of Reduced Universal Adhesive Application Time on Enamel Bond Fatigue and Surface Morphology. Oper Dent. Jun 1. doi: 10.2341/17-261-L.

Naves LZ, Novais VR, Armstrong SR, Correr-Sobrinho L, Soares CJ (2012) Effect of gamma radiation on bonding to human enamel and dentin. Support Care Cancer. Nov;20(11):2873-8.

Novais VR, Soares PB, Guimarães CM, Schliebe LR, Braga SS, Soares CJ (2016) Effect of Gamma Radiation and Endodontic Treatment on Mechanical Properties of Human and Bovine Root Dentin. Braz Dent J. Oct-Dec;27(6):670-674.

Pioch T, Golfels D, Staehle HJ (1992) An experimental study of the stability of irradiated teeth in the region of the dentinoenamel junction. Endod Dent Traumatol. Dec;8(6):241-4.

Qing P, Huang S, Gao S, Qian L, Yu H (2015) Effect of gamma irradiation on the wear behaviour of human tooth enamel. Sci Rep. Jun 23;5:11568.

Reed R, Xu C, Liu Y, Gorski JP, Wang Y, Walker MP (2015) Radiotherapy effect on nano-mechanical properties and chemical composition of enamel and dentine. Arch Oral Biol. May;60(5):690-7.

Rodrigues RB, Soares CJ, Junior PCS, Lara VC, Arana-Chavez VE, Novais VR (2018) Influence of radiotherapy on the dentin properties and bond strength. Clin Oral Investig. Mar;22(2):875-883.

Santin GC, Palma-Dibb RG, Romano FL, de Oliveira HF, Nelson Filho P, de Queiroz AM (2015) Physical and adhesive properties of dental enamel after radiotherapy and bonding of metal and ceramic brackets. Am J Orthod Dentofacial Orthop. Aug;148(2):283-92.

Seyedmahmoud R, Wang Y, Thiagarajan G, Gorski JP, Reed Edwards R, McGuire JD, Walker MP (2018) Oral cancer radiotherapy affects enamel microhardness and associated indentation pattern morphology. Clin Oral Investig. May;22(4):1795-1803.

Soares CJ, Castro CG, Neiva NA, Soares PV, Santos-Filho PC, Naves LZ, Pereira PN (2010) Effect of gamma irradiation on ultimate tensile strength of enamel and dentin. J Dent Res. Feb;89(2):159-64.

Vissink A, Jansma J, Spijkervet FK , Burlage FR, Coppes RP (2003) Oral sequelae of head and neck radiotherapy. Crit Rev Oral Biol Med. 14(3):199–212

Xu C, Reed R, Gorski JP, Wang Y, Walker MP (2012) The Distribution of Carbonate in Enamel and its Correlation with Structure and Mechanical Properties. J Mater Sci.Dec;47(23):8035-8043.

TABLES

Table 1 - Failure mode classification.

Failure mode	Definition
Adhesive failure	Failure occurred entirely within the adhesive area.
Cohesive failure in enamel	Failure occurred predominantly within the enamel area.
Cohesive failure in composite resin	Failure occurred predominantly within the composite resin area.
Mixed failure	Failure continued from the adhesive into either the composite resin or enamel.

Table 2 - Mean bond strength (μSBS) values and standard deviation for the experimental groups.

Experimental groups	Bond strength (MPa)
CT	28.17±6.44 A
IA	30.08±5.97 A
IB	18.68±7.02 B

Upper case letters show statistical difference in vertical ($p < 0.05$)

Table 3- Failure mode analysis of the experimental groups for enamel bonding.

Failure mode	Groups		
	CT	IA	IB
Adhesive failure	14 (50%)	10 (35.71%)	13 (46.43%)
Cohesive failure in enamel	4 (14.28%)	11 (39.29%)	4 (14.28%)
Cohesive failure in composite resin	3 (10.72%)	1 (3.57%)	2 (7.14%)
Mixed failure	7 (25%)	6 (21.43%)	9 (32.15%)
Total	28 (100%)	28 (100%)	28 (100%)

FIGURES

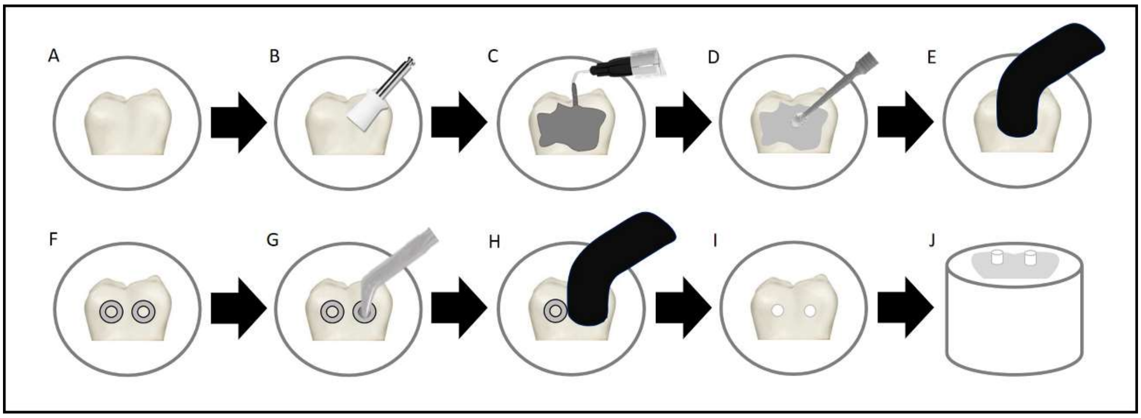

Figure 1 - Schematic representation of specimen preparation for microshear bond strength: A- Crown included in polystyrene resin cylinder; B- Prophylaxis with a rubber cup; C- 37% phosphoric-acid etching; D- Application of the adhesive system; E- Light-curing; F- Tygon tubing placed on the enamel surface; G- Insertion of the composite resin into the tubes; H- Photoactivation of the

composite resin; I- Specimen after removing the tygon tubing; J- Specimen ready for microshear bond test.

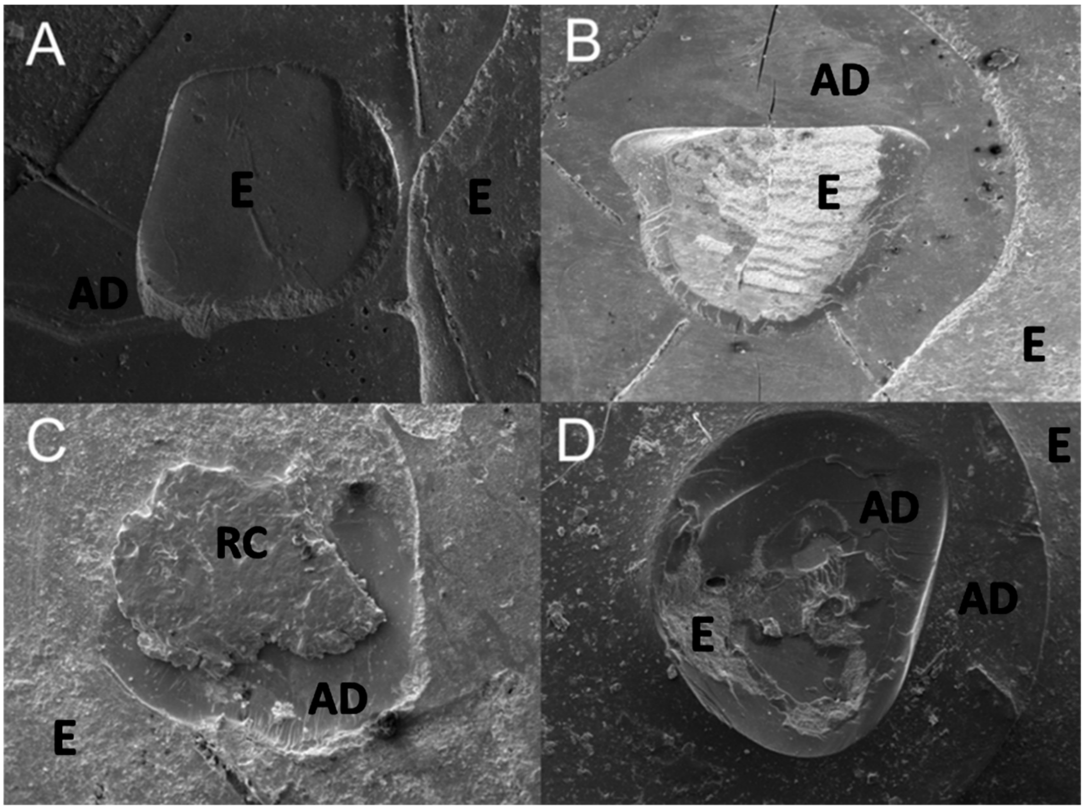

Figure 2 - Failure mode examples of the IB group: A) Adhesive failure; B) Cohesive failure in enamel; C) Cohesive failure in composite resin; D) Mixed failure (AD=adhesive; E=enamel; CR= composite composite).

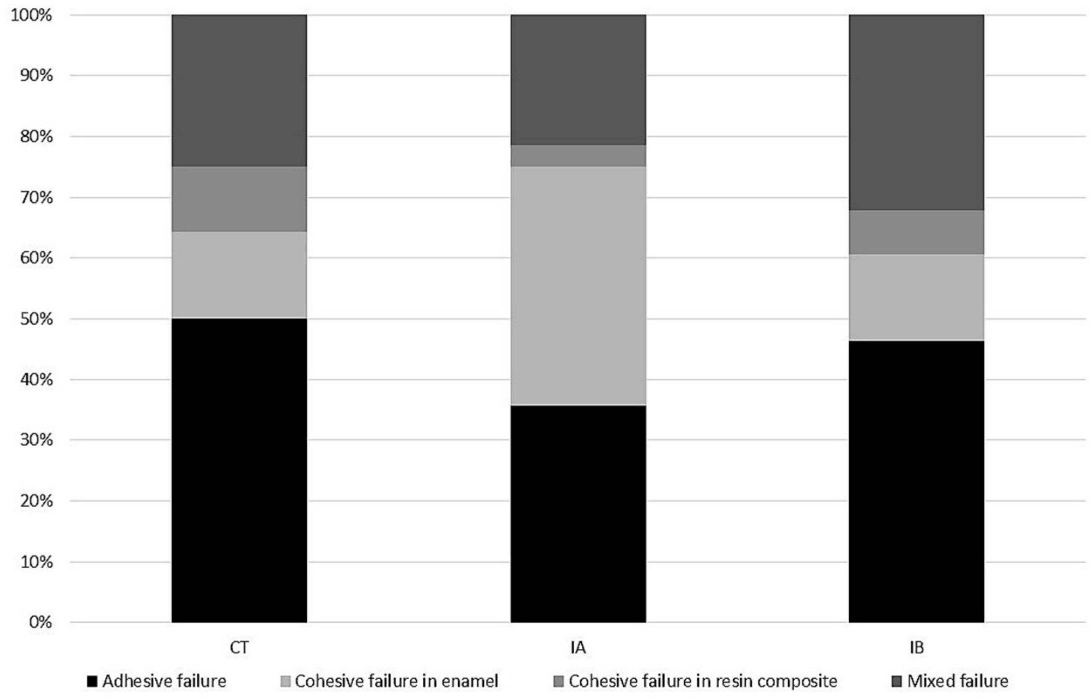

Figure 3 - Failure mode distribution (%) for the experimental groups for enamel bonding.

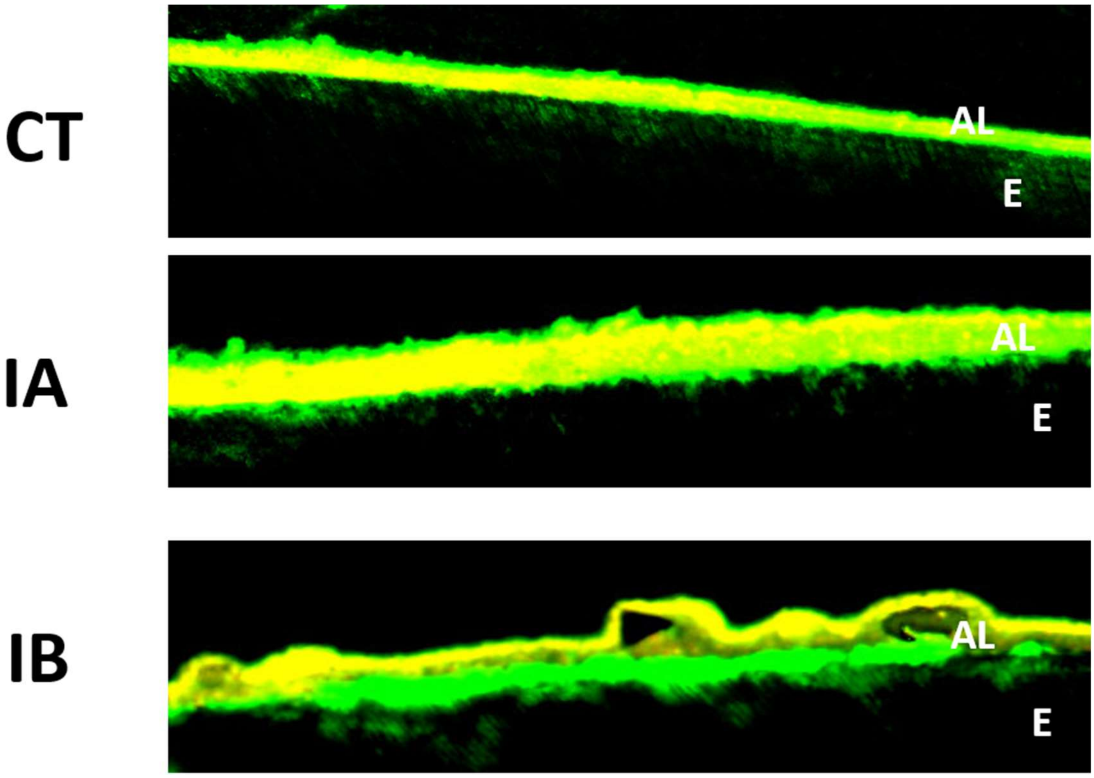

Figure 4 - Adhesive interface formed for each group (Control - CT, IA and IB). It is observed the adhesive layer formed (AL) in all groups.

3.3 Capítulo 3

Impact of radiotherapy in chemical composition and mechanics properties in the cervical human dentin

Artigo a ser enviado para o periódico *Journal of Dentistry*

Impact of radiotherapy in chemical composition and mechanics properties in the cervical human dentin

ABSTRACT

This study evaluated the effect of radiotherapy on the chemical composition and mechanical properties of cervical human dentin. For this, ten third molar teeth were submitted to radiotherapy *in vitro* according to the following radiotherapy protocol: 1.8 Gy daily, 5 days per week during 8 weeks, totaling 72 Gy. The teeth were divided according to the irradiation treatment (n = 5): Control/ non-irradiated group and Irradiated group. For each group, the dentin of the cervical region was evaluated. Its chemical composition was evaluated by Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy using the following parameters: mineral / matrix ratio (M: M), carbonate/mineral ratio (C: M), amide I / amide III ratio and amide I / CH_2 ratio. The mechanical properties (Nanonanohardness and Elastic Modulus) was evaluated by instrumented indentation. T-test ($\alpha < 0.05$) was performed for statistical analysis in each parameter evaluated comparing non-irradiated and irradiated groups. The results showed statistical difference for the FTIR analyzes for the following ration: C:M ($p < 0.004$) and amide I/ amide III ($p = 0.007$). Raman analyzes showed statistical difference for the M:M ratios ($p < 0.001$); amide I/ amide III ($p < 0.001$) and amide I / CH2 ($p < 0.001$). Mechanical evaluation presented difference for nanohardness ($p = 0.04$) and elastic modulus ($p = 0.003$). It can be concluded that the radiotherapy altered the chemical composition and the mechanical properties of the cervical dentine.

Keywords: Biomechanical properties, Dentin, Radiotherapy.

1. INTRODUCTION

Medicine has some types of head and neck cancer treatments, such as those based on irradiation and/or chemotherapy and surgeries [1]. Radiotherapy is an ionizing radiation-based therapeutic approach highly effective in treatment and tumor control; however, causes many adverse reactions that significantly affect the quality of life of patients [1,2]. When applied on the head and neck region, the treatment can cause damage to the salivary glands, mucous membranes, taste buds, bones, and teeth [2]. Signs and symptoms including mucositis, hyposalivation and subsequent xerostomia, osteoradionecrosis, and radiation caries are commonly reported following radiotherapy in this region [2-5].

Scientific evidence suggests that the risk for the development of radiation caries will be present throughout the patients' lives after radiotherapy [3]. This is due to the direct changes that irradiation cause in the dental tissues [5-10]. The ionizing radiations break water molecules in the dental structure. This process generates free radicals of hydrogen and hydrogen peroxide, which are strong oxidation reagents. By acting, they can cause denaturation of the organic components of dental structures [11]. These changes cause damage to dentin collagen fibrils, interprismatic region of enamel altering microhardness, elasticity and fracture resistence [12-16]. It also alters the tensile strength, and stability of the dentin-enamel junction [17,18].

The association of a fragile dental structure with low salivary flow and cariogenic diet makes the dental caries a common disease affecting patients with head and neck cancer [2,5,9], as well as the quality of life of these individuals [19]. The process of glandular degeneration is a consequence of the radiotherapy treatment, resulting in hyposalivation and xerostomia [20]. Considering the importance of saliva for the maintenance of oral health, changes in quantity and quality of saliva make the buccal environment definitely cariogenic [2]. Radiation caries lesions differ from conventional caries in terms of location, appearance, and progression [9]. Post-radiotherapy caries tends to occur in the cervical (junction between crown and root), cuspid, and incisal regions [3,9]. The direct alteration in the enamel and dentin, associated with the salivary alterations, make that the progression of this type of caries is very fast, being able to cause in a short time the rupture of the dental crown.

Is there a correlation between both radiotherapy treatment and dental structure alteration with dental caries on cervical region in neck and head cancer patients? Knowing that one of region most affected by the radiation caries is the cervical region [21], a more specific study in this region becomes important. In this context, the objective of this study was to evaluate the effect of radiotherapy on the chemical composition and mechanical properties of cervical human dentin. The null hypothesis was that radiotherapy will not compromise the chemical composition and mechanical properties of cervical dentin.

2. MATERIALS AND METHODS

After approval from the Ethical Committee in Research (CAAE 60743716.2.0000.5152), ten non-carious human third molars were collected, cleaned and examined using a stereo microscope (Leica MS5; Leica Microscopy Systems Ltd, Heerbrugg, Switzerland) to check caries and another structural defect absence. The teeth were stored in deionized water at 4 ºC and changed weekly, up to 3 months after extraction. The teeth were divided into two groups (n=5), control/ non-irradiated and irradiated. The teeth of irradiated group were submitted to the same radiotherapy protocol applied to patients at the Cancer Hospital of the Federal University of Triangulo Mineiro (Universidade Federal do Triângulo Mineiro – UFTM), with a linear accelerator (Clinac 600C Varian® - Palo Alto, CA, EUA, Beam of 6 MV), 1.8 Gy daily, 5 days per week for 8 weeks, totalizing 72 Gy. During irradiation, the teeth remained immerse in deionized water, changed weekly.

Specimen Preparation

The teeth were cut using a water-cooled diamond saw (Isomet, 15HC diamond; Buehler Ltd., Lake Bluff, IL, USA) mounted on a precision saw (Isomet

1000; Buehler Ltd., LakeBluff,IL, USA), under refrigeration. The first cut was in the cementoenamel junction and, then, 2.5 mm below the junction, to obtain a portion corresponding to the cervical region of the root dentin. Afterwards, the enamel was removed. All slices were cut longitudinally in the mesial-distal direction, resulting in two halves: buccal and lingual. The analyses were performed on the cementoenamel junction surface, of buccal halves with perpendicular dentin tubules. All tests were performed in the same sample. First FTIR and Raman spectroscopies, followed by instrumented nanoindentation. The scheme in Figure 1 summarizes sample preparation and the methodologies proposed.

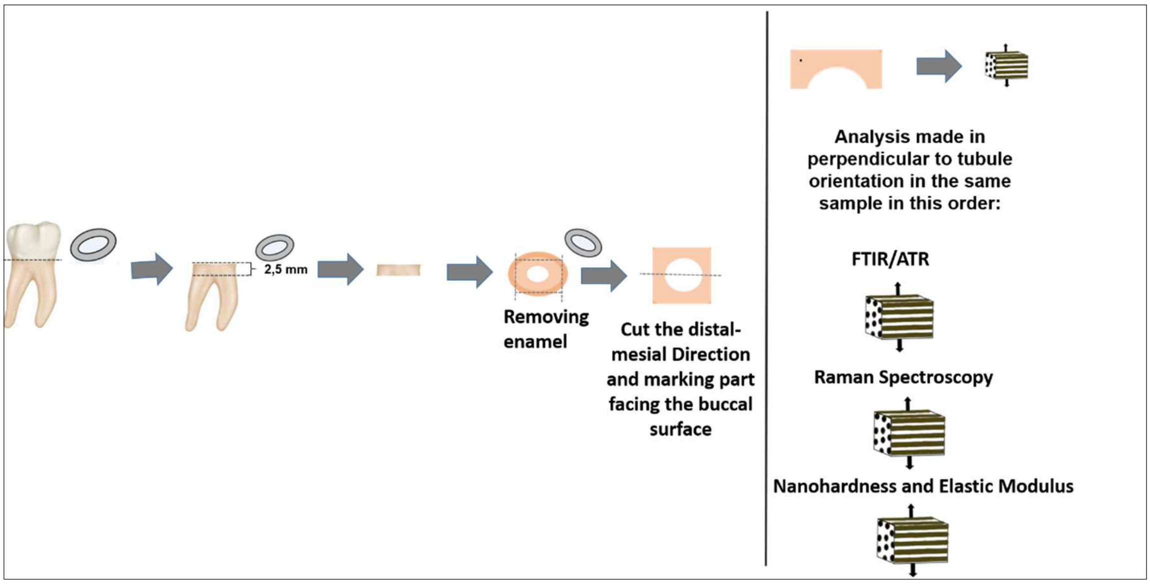

Figure 1. Illustrative scheme of sample cut

Chemical analysis

Fourier Transform Infrared Spectroscopy (FTIR)

The chemical composition of each sample was evaluated using Fourier-transform infrared spectroscopy (FTIR, Vertex 70 - Bruker, Ettlingen, Germany) with attenuated total reflectance (ATR). Each testing surface was carefully positioned against the diamond crystal of the ATR unit, and a constant pressure was applied to facilitate contact. The absorbance spectrum was acquired by scanning the specimens 32 times in the range from 400 to 4000 cm^{-1} at a 4-cm^{-1} resolution and then analyzed by OPUS 6.5 software (Bruker, Ettlingen, Alemanha). After baseline correction and normalization, the FTIR spectra were analyzed calculating the following parameters: (1) mineral/matrix ratio M:M (expressed by the ratio between integrated areas of bands phosphate v_1, v_3 stretching mode – 960cm^{-1} and 1040 cm^{-1}, and of protein amide I – 1655 cm^{-1}; (2) carbonate/mineral ratio C:M (the ratio of the integrated areas of carbonate v_2 at 872 cm^{-1} to the phosphate v_1, v_3); (3) amide I/amide III ratio (the ratio of the integrated areas of amide I at 1,655 cm^{-1} to the amide III at 1,235 cm^{-1}); (4) amide I/CH_2 ratio (the band ratio of the integrated areas of amide I at 1,655 cm^{-1} to the CH_2 scissoring at 1,450 cm^{-1}) [7, 22].

Raman spectroscopy

Raman spectra were obtained by LabRam HR Evolution Raman spectrometer (HoribaLabRam, Villeneuve d'Ascq, France), operating with an excitation power of 20 mW with radiation emitted by a He-Ne laser (632.8 nm). Raman signal is acquired using a grid of 600 lines/mm centered between 300 and 3,100 cm^{-1} with a 400-µm confocal hole. A high-resolution monitor visually indicates the position in which the Raman spectra were collected. The same regions analyzed in the FTIR detection were evaluated by Raman spectroscopy. OriginPro 7.5 software (OriginLab Corporation, Northampton, MA, USA) was used for spectral construction and analysis. The Raman spectra were adjusted by manual correction of multiple baseline. The band at 960 cm^{-1} phosphate vibration v_1 was selected as the internal standard for normalization. Peaks at at 1.655/1.667 cm^{-1}, 1.246/1.270 cm^{-1} and 1.450 cm^{-1} were evaluated corresponding to amide I, amide III and CH_2, respectively. This analysis was used to identify the molecular conformation of the polypeptide chains [23,24]. The mineral components present in hydroxyapatite, peak at 1,070 cm^{-1} is attributed to the v_1 vibration of the carbonate group and peak at 960 cm^{-1} attributed to the v_1 vibration of the phosphate, were also evaluated [23,24].

Based on the Raman spectra, the proportion of the bands of phosphate v_1 to amide I were calculated to analyze the differences in the mineral / matrix relation (M: M) of specimens. The proportion of carbonate with band at 1.070 cm^{-1} with phosphate band at 960 cm^{-1} was obtained to analyze differences in mineral composition (C: M). To determine the nature of collagen in the specimens, the following indexes was calculated: (1) amide I / amide III regardings collagen organization; and (2) amide I / CH2 indicating change in collagen quality [24].

Mechanical Analysis

Nanohardness and Elastic Modulus

The specimens were embedded in an acrylic resin for polishing. The surfaces were polished with silicon-carbide abrasive papers (#600, 800, 1200, 1500 and 2000 grit sizes; Norton, Campinas, SP, Brazil) with constant water irrigation and polished with felt discs and metallographic diamond pastes (6-, 3-, 1-, and ¼-µm sizes; Arotec, São Paulo, SP, Brazil). The specimens were washed with deionized water and cleaned ultrasonically in distilled water for 5 minutes between each metallographic diamond paste polishing procedure.

Nanohardness and elastic modulus were measured by an instrumented nanoindenter UNAT - (ASMEC - Zwick Roell Group, Germany), using a diamond pyramidal tip (Berkovich type). Its area function was calibrated using fused silica and sapphire reference samples. The maximum load of the indentation tests increased quadratically up to 15 mN, with a dwell time of 30 s. The average maximum depth was about 1 μm. The indentations were performed at 12 locations where there were any dentin tubules so they would not affect the nanohardness and elastic modulus measurements. The indentations were50 µm apart from each other. By monitoring simultaneously, the load, penetration depth and the surface elastic recovery after unloading, nanohardness and elastic modulus were calculated in a nanometric scale using the InspectorX software (ASMEC, Germany) using the Oliver-Pharr method [25]

Statistical analysis

Data were tested for normal distribution (Shapiro-Wilk, $p>0.05$) and equality of variances (Levene's test, $p>0.05$). Chemical and mechanical properties were analyzed with T-test considering the factor irradiation. Sigma Plot statistical software (version 12.0, Systat Software, Inc., San Jose, CA, USA) was used for analysis and $\alpha<0.05$ was considered to be statistically significant.

3. RESULTS

Chemical analysis

ATR/FTIR

The mean of the integrated areas of each band observed in the FTIR spectra are shown in Table 1. Means and standard deviation for ratio of the parameters obtained by FTIR spectra are shown in Table 2. There was no statistical difference for M:M ratio, ($p=0.255$) and amide I/CH_2 ratio ($p=0.918$). For C:M and Amide I/amide III ratios, the irradiation factor was significant ($p=0.004$ and $p=0.007$, respectively). It was observed for both ratios that the irradiated group presented lower values than the control group.

Table 1. Mean of the integrated areas of each band corresponding to the chemical component in the FTIR spectra

	Amide I	Amide III	CH2	Phosphate	Carbonate
Control	1.43	0.19	0.15	13.35	0.37
Irradiated	1.62	0.25	0.17	13.62	0.31

Table 2. Means and standard deviation for the ratios of the bands corresponding to the chemical component in the FTIR spectra.

	M:M Ratio Phosphate/ Amide I	**C:M Ratio Carbonate/ Phosphate**	**Amide I/ Amide III**	**Amide I/CH_2**
Control	9.55 (1.78) A	0.028 (0.001) A	7.35 (0.46) A	9. 60(1.06) A
Irradiated	8.44 (0.95) A	0.022 (0.002) B	6.04 (0,66) B	9.51 (1.54) A

Different letters show statistical difference in vertical

Raman Spectroscopy

The mean of the integrated areas of each band evaluated by Raman Spectroscopy is shown in Table 3. It is observed that the irradiated group presented higher values than the control groups for the organic components and a stability values for the inorganic components. The means and standard deviation for the ratio of the parameters are shown in Table 4. The M:M ratio showed statistical difference ($p<0.001$), while for C:M ratio, the irradiation was not significant ($p=0.987$). For the Amide I / Amide III and Amide I / CH_2 ratios there was a statistical difference between control/no irradiated e irradiated groups, with ($p<0,001$) for both.

Table 3. Means of the integrated areas of each Raman band corresponding to the chemical component in the Raman spectra.

	Amide I	**Amide III**	**CH2**	**Phosphate**	**Carbonate**
Control	0.07	0.05	0.07	1	0.17
Irradiated	0.12	0.07	0.06	1	0.17

Table 4. Means and standard deviation for the ratios of the of the bands corresponding to the chemical component in the Raman spectra.

	M:M Ratio Phosphate/ Amide I	C:M Ratio Carbonate/ Phosphate	Amide I/ Amide III	Amide I/CH_2
Control	14.28 (1.38) A	0.175 (0.004) A	1.29 (0.10) B	0.98 (0.014) B
Irradiated	8.33 (0.63) B	0.175 (0.009) A	1.71 (0.16) A	2.10 (0.12) A

Different letters show statistical difference in vertical

Mechanical analysis

Nanonanohardness and Elastic Modulus

The mean values and standard deviation of the Nanohardness and Elastic Modulus are shown in Table 5. There was a statistical difference for Nanohardness (p=0.04) and elastic modulus (p=0.003), where the irradiated group showed lower values for both properties.

Table 5. Means and standard deviation for Nanohardness and Elastic Modulus

	Nanohardness (Gpa)	Elastic Modulus (GPa)
Control	0.59 (0.02) A	20.6 (0.6) A
Irradiated	0.51 (0.1) B	17.8 (1.3) B

Different letters show statistical difference in vertical

4. DISCUSSION

The null hypothesis was rejected, since the radiotherapy changed the chemical composition and mechanical properties of dentin in the cervical region. Several researchers seek to understand the mechanism involved in the rapid progression of radiation caries [3, 5, 7-18; 26,27] The involvement of the cervical region is of concern because of the rapid possibility of loss of the dental crown. In this region, caries lesion starts from the buccal surface up to the lingual surface, then progressing around the tooth as an annular lesion [3]. Literature shows that oral cancer patients exposed to radiotherapy demonstrate post-radiation dental lesions that initiate with enamel shear fracture that can result in partial to total enamel delamination, suggesting instability of dentin-enamel junction (DEJ) [11, 17, 28]. This loss of enamel may be related to collagen alteration, in teeth submitted to radiation therapy, since this represents an instability mechanism of the DEJ [30]. All these data justify the fragility of the cervical region. With the pathologic enamel loss, there is an exposure of the cervical dentin with a consequent rapid progression of the radiation caries [9]. The results obtained in the present study showed a direct damage caused by radiotherapy in dentin of this region.

In this study, the teeth were irradiated *in vitro*, since direct radiation on teeth allows an isolated evaluation of the effects caused on chemical composition and mechanical behavior of dentin, without interferences such as pH of saliva, xerostomia, and patients' diet [2, 6].

The FTIR and Raman Spectroscopic techniques were used by investigate lhe chemical analysis based on the bands observed in the spectra that are attributed to the vibrational modes of the compounds. Thus, one can investigate changes in the composition of the dentin specimens subjected to radiotherapy. Both techniques are able to evaluate organic and inorganic compounds; however, the way to detect the chemical molecules and bonds are different [30]. The FTIR is based on light absorption effects, so this technique has a small and well controlled penetration depth, measuring the specimen layer that is in contact with the ATR crystal [31]. This spectroscopic technique refers to the absorption of infrared radiation and depends on the energy absorption of a photon that subsequently promotes the transition from a low energy vibrational state to a higher energy or excited vibrational state [22,30]. Raman spectroscopy is regulated by processes of light scattering by matter [22, 30]. A change in the polarization of the molecules is observed; that is, a visible or ultraviolet photon interacts with the vibrating molecular bonds, gaining or losing some of its energy, thus generating the spectrum [22, 30]. On Raman spectroscopy, the spectral analysis is performed in light scattering and reflection mode, which allows to analyze the samples in greater depth [31]. Therefore, FTIR and Raman are complementary techniques [7].

In the FTIR spectra observed a significant change in the bands of inorganic portion of dentin. Significantly lower values were observed for the irradiated group in the C: M ratio (Table 2). This occured due to the lower value of the area of the carbonate band of the irradiated group in relation to the control group (Table 1). Carbonate/ phosphate bands ratio indicates the extent of carbonate incorporation into the hydroxyapatite lattice and shows whether there is phosphate replacement of carbonate [32]. The results obtained for this analysis show a decrease in the carbonate/ phosphate bands ratio, indicating that the radiotherapy was able to act in the mineral matrix. The literature shows that there is a molecular rearrangement, and the carbonate may replace phosphate ions in the dentin hydroxyapatite, forming a carbonated hydroxyapatite [33, 34]. This fact may justify the lower values presented by the carbonate band, considering that what happens is a substitution of this molecule. This substitution causes deformations in the hydroxyapatite crystalline lattice and results in less stable phases that may be more soluble in acid [35]. These results corroborate with the findings of [7], which also showed a decrease in the C: M ratio in irradiated root dentin *in vivo* using FTIR technique. Using another methodology was showed a decrease in the Ca / P weight ratio in irradiated radicular dentin *in vitro* [26], and this ratio is related to the rate of mineralization of hydroxyapatite [36]. This is an important parameter because both the mechanical properties of the dental substrate and its biodegradation rate depend strongly on the hydroxyapatite mineralization pattern [36]. Thus, it is suggested that the radiation employed in the present study, altered the mineralization pattern of the dentin hydroxyapatite, probably making it more soluble. This change may contribute to the dentinal degradation observed in the radiation caries, and favors the rapid progression of the lesion.

In relation to the changes in the organic matrix, FTIR and Raman spectroscopy techniques presented statistical significance for the Amide I/ Amide III bands ratio. Both showed higher values for band area of amide I and amide III to irradiated groups (Table 1 and Table 3). A similar result was observed when evaluating the effect of radiotherapy on coronary and root dentin, respectively [7, 8]. However, in assessing the ratio, different results can be observed. FTIR presented a lower value while the Raman presented a higher value of the irradiated group (Table 2 and Table 4). This ratio is related to the organization of collagen [24]. Amide I is the most intense absorption range in proteins and is primarily governed by the stretching vibrations of the C = O (70-85%) and C - N (10-20%) groups [37]. Amide III is a very complex band, dependent on the details of the force field, the nature of the side chains and the hydrogen bond [37].

As previously explained, chemical composition analyzes by FTIR and Raman spectroscopy the result of different physical phenomena. Both techniques showed increased values of bands area of amide I and amide III. However, Raman spectroscopy showed greater changes, since the percentage difference between integrated areas between the control and irradiated groups was higher. Considering that the analysis was performed in the same sample, it can be suggested that because the Raman analysis reached a greater depth inside the sample, it was able to detect larger differences. This difference in the detection of each molecule made the behavior of the ratios different. However, both techniques showed that there was a change in the organization of collagen. An important characteristic of the collagen present in dentin is the expressive number of intermolecular covalent crosslinks between the chains, as a result of the interaction between aldehyde groups and free amino groups. Such crosslinking provides the stability and tensile strength required by the structure [38]. It has already been shown in other studies that free radicals formed by radiotherapy in the presence of water break down the side hydrogen bonds of the collagen molecule, altering its conformation [39]. Such free radicals can further interact with the terminal molecules in the collagen structure, causing a structural rearrangement in the molecule producing an increase in the amounts of amide I and amida III [7].

Raman spectroscopy also showed significant alteration for the Amide I/ CH_2 bands ratio (Table 4), which is related to collagen quality and for the M: M bands ratio (Table 4). This also happened because of the greater area value of the Amida I and CH_2 bands for the irradiated group. A relative increase of area bands of CH_2 and amide I can indicates altered collagen quality induced by aging, hydration/dehydration or radiological damage [17, 24].

The results of the chemical analyze confirm the fragility of the dentin of the cervical region. Changes in the organic portion alter the conformation of the collagen molecule and probably contribute to the degradation of the dentin tissue. Such changes caused directly by radiotherapy, associated with the indirect side effect mainly as hyposalivation, make the oral environment highly cariogenic, being probably able to favor the rapid evolution of the caries lesion. The changes in the organic matrix can also be related to the activation of collagen-degrading metalloproteinases [27]. Although it has not been the objective of the present study, it is reported in the literature that radiotherapy can activate collagenolytic enzymes and this activation can contribute to the progression of the radiation related-caries [27].

In addition to the chemical composition, the present study also evaluated the mechanical properties of cervical dentin. Several papers showed a decrease in dentin hardness when undergoing radiotherapy [6, 12, 14, 26]. High-energy irradiation can alter the mineral and organic matrix, and this alteration becomes more severe with increasing radiation dosage [12, 26]. These studies associate the reduction of nanohardness after radiotherapy with collagen degradation, micromorphological changes, and the formation of less structured hydroxyapatite crystals [12]. These structural defects can make the dentin dry and brittle [12], which also decreases its mechanical resistance.

The mechanical properties of dentin describe the response of the tooth to masticatory loads. The nanohardness and elastic modulus are examples of such properties and reflect complex interactions of tissue microstructure [40]. Human dentin is a complex tissue composed of inorganic matrix (hydroxyapatite crystals) and organic matrix [41]. The mineral occupies two sites within this collagen scaffold: intrafibrillar (inside the periodically spaced gap zones in the collagen fibril) and extrafibrillar (in the interstices between the fibrils) [42, 43]. A distinct feature of collagen is its cross-linking chemistry and molecular packing structure. This arrangement is responsible for mechanical properties such as tensile strength and viscoelasticity of the fibrillar matrix [44]. Considering that the present study evaluated the chemical composition and later the mechanical properties of the same sample, it can be stated that the alteration of the mineralization pattern of hydroxyapatite, associated with changes in the structure of the collagen molecule caused the reduction of the values of nanohardness and elastic modulus. It is known that radiotherapy causes a decarboxylation of the side links of carboxylate in collagen and this link is responsible for the interaction of mineral matrix with hydroxyapatite crystals [39]. The decrease of the mechanical properties can also be related to this decrease of the mineral-organic interaction.

The results of this study are of great importance as they show the vulnerability of the dentin of the cervical region. Chemical alterations may be related to a remarkable disorganization of the apatite crystals [26], associated with the changes observed in the molecules of collagen proteins (Amida I, II and III) [7]. These changes are directly related to the dental biomechanical behavior, shown in this article by the significant reduction of nanohardness and elastic modulus. These mechanical changes also reflect direct damage from radiotherapy, which weakens the dental element, contributing to early dental fractures and loss.

These data should always challenge researchers so that they can continually seek ways to minimize these harmful radiation effects and create efficient preventive and restorative alternatives to rehabilitate those patients. Longitudinal follow-up clinical studies are needed to better demonstrate the risks of these complications associated with external factors, such as changes in patients' diet, oral pH and hyposalivation. All these factors contribute to the development of radiation related-caries, and dental structural alteration enhances the development of the disease, causing severe destruction of the teeth. In addition, new studies that allow a deeper understanding of changes in the cervical region, as well as a mapping of these changes in all dental regions, can help in the search for more predictive restorative and preventive protocols. Medicine has advanced and the cure of cancer is a reality. Only the continuous search for knowledge can improve the quality of life of patients who survive oncological disease, but they have to cope with the sequelae of radiotherapy.

5. CONCLUSIONS

With the accomplishment of this work it can be concluded that radiotherapy altered the mineralization pattern of the hydroxyapatite, was able to change the organization and quality of the collagen and altered the mechanical properties of the cervical dentin.

ACKNOWLEDGEMENTS

This research was supported by the Minas Gerais State Agency for Research and Development (FAPEMIG), National Council for Scientific and Technological Development (CNPq) and Coordination of Personal Development at a Higher Level (CAPES).

The authors are indebted the Radiotherapy Sector of the Federal University of Triangulo Mineiro for the irradiation of the samples.

The authors are grateful to C-LABMU/UEPG for the use of the laboratory facilities.

6. REFERENCES

1. F. De Felice, A. Polimeni, V. Valentini, O. Brugnoletti, A. Cassoni, A. Greco, M. de Vincentiis, V. Tombolini. Radiotherapy Controversies and Prospective in Head and Neck Cancer: A Literature-Based Critical Review. Neoplasia. (2018) Mar;20(3):227-232.
2. H. Jawad, N.A Hodson, P.J Nixon. A review of dental treatment of head and neck cancer patients, before, during and after radiotherapy: part 1. Br Dent J (2015) 218(2):65-8.
3. A.M Kielbassa, W Hinkelbein, E Hellwig, H Meyer-Luckel Radiation-related damage to dentition Lancet Oncol (2006) 7:326-335 doi:10.1016/S1470-2045(06)70658-1

4. J.H Meurman, L. Gronroos L. Oral and dental health care of oral cancer patients: hyposalivation, caries and infections Oral Oncol (2010) 46:464-467 doi:10.1016/j.oraloncology.2010.02.025
5. N. Gupta, M Pal, S. Rawat, M.S Grewal, H. Garg, D. Chauhan, P. Ahlawat, S. Tandon, R. Khurana, A.K. Pahuja, M. Mayank, B. Devnani. Radiation-induced dental caries, prevention and treatment - A systematic review Natl J Maxillofac Surg (2015) 6:160-166 doi:10.4103/0975-5950.183870
6. H.F.L Lieshout & C.P Bots. The effect of radiotherapy on dental hard tissue—a systematic review. Clin Oral Investig (2014) 18:17–24.
7. R.R de Miranda, A.C.A. Silva, N.O. Dantas, C.J. SoareS, V.R. Novais Chemical analysis of in vivo-irradiated dentine of head and neck cancer patients by ATR-FTIR and Raman spectroscopy Clinical oral investigations (2018) doi:10.1007/s00784-018-2758-6
8. R.B. Rodrigues, C.J. Soares, P.C. Simamoto Junior, V.C. Lara, V.E. Arana-Chavez, V.R. Novais. Influence of radiotherapy on the dentin properties and bond strength. Clin Oral Investig. (2018) 22(2):875-883.
9. M.P. Walker, B. Wichman, A.L. Cheng, J. Coster, K.B. Williams. Impact of Radiotherapy Dose on Dentition Breakdown in Head and Neck Cancer Patients. Pract Radiat Oncol. (2011) 1(3):142-148.
10. H. Lu, Q. Zhao, J. Guo, B. Zeng, X. Yu, D. Yu, W. Zhao. Direct radiation-induced effects on dental hard tissue Radiation oncology (2019) 14:5 doi:10.1186/s13014-019-1208-1
11. T. Pioch, D. Golfels, H.J. Staehle. An experimental study of the stability of irradiated teeth in the region of the dentinoenamel junction. EndodonticDental Traumatology (1992) 8:241-244.
12. L.M. Gonçalves, R.G. Palma-Dibb, F.W. Paula-Silva, H.F. Oliveira, P. Nelson-Filho, LA. Silva, et al. Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth. J Dent. (2014) 42(8):986-92.
13. P. Qing, S. Huang, S. Gao, L. Qian, H. Yu. Effect of gamma irradiation on the wear behaviour of human tooth enamel Scientific reports (2015) 5:11568 doi:10.1038/srep11568

14. P. Qing, S. Huang, S. Gao, L. Qian, H. Yu. Effect of gamma irradiation on the wear behavior of human tooth dentin Clinical oral investigations (2016) 20:2379-2386 doi:10.1007/s00784-016-1731-5
15. X. Liang, J.Y. Zhang, I.K. Cheng, J.Y. Li JY Effect of high energy X-ray irradiation on the nano-mechanical properties of human enamel and dentine Brazilian oral research (2016) 30 doi:10.1590/1807-3107BOR-2016.vol30.0009
16. C.C.A. Lopes, C.J. Soares, V.C. Lara, V.E. Arana-Chavez, P.B. Soares, V.R. Novais. Effect of fluoride application during radiotherapy on enamel demineralization. J Appl Oral Sci. (2018) doi: 10.1590/1678-7757-2018-0044.
17. R. Reed, C. Xu, Y. Liu, J.P. Gorski, Y. Wang, M.P. Walker, Radiotherapy effect on nano-mechanical properties and chemical composition of enamel and dentine,Arch Oral Biol. (2015) 60(5) 690-697.
18. C.J. Soares, C.G. Castro, N.A. Neiva, P.V. Soares, P.C.F. Santos-Filho, L.Z. Naves, et al. Effect of gamma irradiation on ultimate tensile strength of enamel and dentin. J Dent Res. (2010) 89(2):159-64.
19. B. Al-Nawas, K. Al-Nawas, M. Kunkel, K.A. Grötz. Quantifying radioxerostomia: salivary flow rate, examiner's score, and quality of life questionnaire.Strahlenther Onkol. (2006) Jun;182(6):336-41.
20. S.C. Lin, Y.M. Jen, Y.C. Chang, C.C. Lin. Assessment of xerostomia and its impact on quality of life in head and neck cancer patients undergoing radiotherapy, and validation of the Taiwanese version of the xerostomia questionnaire. J Pain Symptom Manage. (2008) Aug;36(2):141-8.
21. A. Vissink, J. Jansma, F.K. Spijkervet, F.R. Burlage, R.P. Coppes. Oral sequelae of head and neck radiotherapy. Crit Rev Oral Biol Med. (2003) 14(3):199-212.
22. C.C.A. Lopes, P.H.J.O. Limirio, V.R. Novais, P. Dechichi. Fourier transform infrared spectroscopy (FTIR) application chemical characterization of enamel, dentin and bone. Appl Spectrosc (2018) Rev 53: 747–769.
23. C. Xu, Y. WanG. Cross-linkeddemineralizeddentinmaintains itsmechanical stability when challenged bybacterial collagenase.J Biomed Mater Res B Appl Biomater (2011) 96(2):242–248 20.

24. M.Toledano, F.S. Aguilera, E. Osorio, I. Cabello, M. Toledano-Osorio, R. Osorio. Functional and molecular structural analysis of dentine interfaces promoted by a Zn-dopedself-etching adhesive and na in vitro load cycling model. J Mech Behav Biomed Mater (2015) 50:131–149
25. W.C. Oliver, G.M. Pharr. An improved technique for determining nanohardness and elastic modulus using load and displacement sensing indentation experiments, Journal of Materials Research. 7 (1992) 1564–1583. doi:10.1557/JMR.1992.1564..
26. M.M.A.C. Velo, A.L.H. Farha, P.S. da Silva Santos, A. Shiota, S.Z. Sansavino, A.T. Souza, H.M. Honório, L. Wang. Radiotherapy alters the composition, structural and mechanical properties of root dentin in vitro. Clin Oral Investig. (2018) Nov;22(8):2871-2878.
27. A.M. Queiroz, C.M.C. Bonilla, R.G. Palma-Dibb, H.F. Oliveira, P. Nelson-Filho, L.A.B. Silva, M.P. Lucisano, F.W.G. Paula-Silva. Radiotherapy Activates and Protease Inhibitors Inactivate Matrix Metalloproteinases in the Dentinoenamel Junction of Permanent Teeth. Caries Res. (2018) Sep 26;53(3):253-259.
28. G.Thiagarajan, B. Vizcarra, V. Bodapudi, R. Reed, R. Seyedmahmoud, Y. Wang, J.P. Gorski, M.P. Walker. Stress analysis of irradiated human tooth enamel using finite element methods. Comput Methods Biomech Biomed Engin. (2017) Nov;20(14):1533-1542.
29. J.D. McGuire, J.P. Gorski, V. Dusevich, Y. Wang, M.P. Walker. Type IV collagen is a novel DEJ biomarker that is reduced by radiotherapy. J Dent Res. (2014) Oct;93(10):1028-34. doi: 10.1177/0022034514548221.
30. J. Zieba-Palus, M. Kunicki. Application of the micro-FTIR spectroscopy, Raman spectroscopy and XRF method examination of inks. Forensic Sci Int (2006) 158(2–3):164–172
31. K.L. Andrew Chan, S.G. Kazarian. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) imaging of tissues and live cells. Chem Soc Rev (2016) 45(7):1850–1864

32. H. Ou-Yang, E.P. Paschalis, W.E. Mayo, A.L. Boskey, R. Mendelsohn. Infrared microscopic imaging of bone: spatial distribution of CO3(2-). J Bone Miner Res (2001) 16(5):893–900.
33. R.Z. LeGeros, C.B. Bleiwas, M. Retino, R. Rohanizadeh, J.P. LeGeros. Zinc effect on the in vitro formation of calcium phosphates: relevance to clinical inhibition of calculus formation. Am J Dent. (1999) Apr;12(2):65-71.
34. C. Rey, B. Collins, T. Goehl, I.R. Dickson, M.J. Glimcher. The carbonate environment in bone mineral: a resolution-enhanced Fourier transform infrared spectroscopy study. Calcif Tissue Int (1989) 45(3):157–164
35. Y. Liu, C.Y. Hsu.Laser-induced compositional changes on enamel: a FT-Raman study. J Dent (2007) 35(3):226–230
36. A. Slosarczyk, J. Piekarczyk. Ceramic materials on the basis of hydroxyapatite and tricalcium phosphate. Ceram Int (1999) 25(6):561– 565.
37. C. Xu, Y. Wang. Chemical composition and structure of peritubular and intertubular human dentine revisited. Arch Oral Biol (2012) 57(4):383–391
38. M. Yamauchi, M. Sricholpech. Lysine post-translational modifications of collagen. Essays Biochem. (2012) 52:113-33. doi: 10.1042/bse0520113.
39. W. Franzel, R. Gerlach, H.J. Hein, H.G. Schaller. Effect of tumor therapeutic irradiation on the mechanical properties of teeth tissue Z Med Phys (2006) 16:148-154
40. D.E. Pashley. Dentin: a dynamic substrate—a review. Scanning Microsc (1989) 3:161-176.
41. I.A. Mjör. Human coronal dentine: structure and reactions. Oral Surg Oral Med Oral Pathol. (1972) May;33(5):810-23.
42. L.C. Bonar, S. Lees, H.A. Mook. Neutron diffraction studies of collagen in fully mineralized bone. J Molec Biol (1985) 181:265-27
43. R.M. Pidaparti, A. Chandran, Y. Takano, C.H. Turner. Bone mineral lies mainly outside collagen fibrils: predictions of a composite model for osteonal bone. J Biomechan (1996) 29:909-916
44. L. Knott, A.J. Bailey. Collagen cross-links in mineralizing tissues: a review of their chemistry, function, and clinical relevance. Bone. (1998) Mar;22(3):181-7.

3.4 Capítulo 4

Manejo odontológico de pacientes oncológicos de cabeça e pescoço: evidência científica com ênfase clínica na cárie relacionada à radiação

Artigo a ser enviado para o periódico *Revista da ABO*

Manejo odontológico de pacientes oncológicos de cabeça e pescoço: evidência científica com ênfase clínica na cárie relacionada à radiação

Dental management of head and neck cancer patients with emphasis on radiation-related caries

RESUMO

A maioria dos pacientes diagnosticados com neoplasias malignas na região de cabeça e pescoço são tratados por meio da radioterapia. É um tratamento bastante eficaz para erradicar células cancerígenas, no entanto, pode acometer tecidos sadios próximos ao tumor. Isso se torna mais evidente quando o campo de irradiação envolve a cavidade oral, podendo atingir glândulas salivares, tecidos moles, ossos e dentes. Como consequências clínicas do acometimento dessas estruturas pela radiação o paciente pode apresentar hipossalivação, xerostomia, mucosite, disgeusia, necrose de tecidos moles e duros e cárie relacionada à radiação (CRR). A CRR é uma doença multifatorial e com potencial altamente destrutivo, que compromete a qualidade de vida e função oral do paciente após o tratamento radioterápico. Seu tratamento é bastante desafiador e o paciente necessita de cuidados específicos. Por isso, é de fundamental importância o conhecimento do cirurgião-dentista sobre as especificidades do controle e tratamento da CRR. Nesse contexto, o presente trabalho apresenta uma discussão dos principais fatores relacionados ao desenvolvimento da CRR, assim como as dificuldades e limitações encontradas no seu tratamento e controle.

Palavras-chave: cárie dentária, dentina, esmalte, radioterapia.

ABSTRACT
Most patients diagnosed with malignant neoplasms in the head and neck region are treated by radiotherapy. It is a very effective treatment to eradicate cancer cells, however it can affect healthy tissues near the tumor. This becomes more evident when the irradiation field involves the oral cavity, which can reach salivary glands, soft tissues, bones and teeth. As a clinical consequence of the involvement of these structures by radiation, the patient may present hyposalivation, xerostomia, mucositis, dysgeusia, soft and hard tissue necrosis, and radiation-related caries (RRC). RRC is a multifactorial disease with a highly destructive potential that compromises the patient's quality of life and oral function after radiotherapy treatment. Its treatment is very challenging and the patient needs specific care. Therefore, it is fundamental the knowledge of the dental surgeon about the specificities of the control and treatment of RRC. In this context, the present study presents a discussion of the main factors related to RRC development, as well as the difficulties and limitations found in its treatment and control.

Keywords: dental caries, dentin, enamel, radiotherapy.

INTRODUÇÃO

Câncer de cabeça e pescoço é o nome dado a um grupo heterogêneo de neoplasias malignas que acometem vários locais, incluindo nasofaringe, seios paranasais, cavidade oral, orofaringe e laringe[1]. Classifica-se como câncer de cavidade oral aqueles que tenham como localização primária os lábios, a cavidade oral, as glândulas salivares e a orofaringe (C00-C10). No Brasil, estima-se um aumento considerável no número de homens e mulheres que serão acometidos pelo câncer na cavidade oral no biênio 2018/2019, sendo, portanto, uma preocupação para a saúde pública[2].

A radioterapia é uma abordagem terapêutica baseada no uso da radiação ionizante como tratamento de neoplasias malignas de forma exclusiva ou associada à cirurgia e/ou quimioterapia. É um tratamento altamente eficaz para o controle de tumores, mas pode provocar reações adversas que afetam significativamente a qualidade de vida dos pacientes[3]. Na cavidade oral, essas reações podem incluir mucosite, xerostomia, perda do paladar, trismo, perda progressiva de inserção periodontal, necrose de tecido mole, osteorradionecrose e cárie dentária[3]. Atualmente, métodos radioterapêuticos como a radioterapia de intensidade modulada (IMRT) são preconizados, buscando um direcionamento mais específico dos raios ionizantes apenas para a região tumoral[4]. No entanto, essa não é a realidade de muitos centros de tratamento oncológico no Brasil[2], que acabam tendo como opção de equipamentos os aparelhos radioterápicos cuja radiação alcança também os tecidos sadios e estruturas nobres da cavidade oral, como glândulas salivares, osso, tecidos moles e dentes[3,5].

A cárie relacionada à radiação (CRR) é a cárie que acomete os pacientes submetidos à radioterapia na região de cabeça e pescoço, quando o campo de irradiação envolve a cavidade oral[3,6]. Assim como a cárie convencional, a CRR possui etiologia multifatorial. A hipossalivação, causada por danos da radiação às glândulas salivares, faz com que o ambiente oral se torne mais ácido[7]. Como consequência da alteração na quantidade e na qualidade da saliva, os pacientes pós-radioterapia podem apresentar desconforto oral e com isso optar por uma dieta rica em alimentos macios. Dessa forma, o meio oral se torna altamente cariogênico: pouca saliva, ambiente ácido e alimentação rica em carboidratos[3].

Além disso, as propriedades químicas e mecânicas dos dentes atingidos pela radioterapia são evidentemente alteradas resultando em enfraquecimento e destruição dos mesmo[8-15]. Desta forma, a cárie desenvolvida nesses pacientes evolui mais rapidamente, podendo acarretar em perda do elemento dentário em pouco tempo.

Portanto, o manejo e o tratamento do paciente após a radioterapia tornam-se extremamente desafiadores para os cirurgiões-dentistas. Neste contexto, o objetivo deste trabalho é apresentar uma discussão dos principais fatores relacionados ao desenvolvimento da CRR, assim como as dificuldades e limitações encontradas no seu tratamento e controle.

Fatores predisponentes da cárie relacionada à radiação (CRR)

A cárie dentária é uma das doenças mais prevalentes em todo o mundo com causa multifatorial, que envolve acúmulo de biofilme, exposição à açúcares e fluoretos, e fatores biológicos e sociais[16]. A associação destes fatores determina ora a desmineralização, ora a remineralização da estrutura dental e caso ocorra algum desequilíbrio leva ao desenvolvimento e progressão da doença[16]. A CRR apresenta os mesmos fatores etiológicos, no entanto, todos os fatores apresentam-se intensificados nos pacientes pós radioterapia. A hipossalivação induzida por radiação, mudanças na dieta, e alterações das propriedades do esmalte e dentina são consideradas os fatores etiológicos dessas lesões[3,5,17,18].

A seguir são apresentados como cada fator contribui para o desenvolvimento desse tipo de cárie.

Alterações Salivares

A maioria dos planos de tratamento estabelecidos para pacientes oncológicos de cabeça e pescoço baseiam-se numa dose total de 50 a 70 Gy, fracionadas em 1,8 a 2 Gy diários, num período de 5 a 7 semanas[19]. Caso as glândulas salivares se encontrem localizadas no campo de irradiação, estas poderão sofrer danos diretos nas suas células glandulares, resultando em uma drástica redução do fluxo salivar[20,21,22]. A diminuição efetiva da quantidade do fluxo salivar é denominada hipossalivação, enquanto que a xerostomia é a sensação subjetiva de boca seca, consequente ou não da diminuição da função das glândulas salivares. A xerostomia começa no início do tratamento radioterápico, quando utilizada dose superior a 40 Gy[20]. Depois de sete semanas de radioterapia convencional, o fluxo salivar diminui aproximadamente 20%[21]. Depois de 12 a 18 meses do término da radioterapia, a recuperação da função salivar pode ou não ocorrer, dependendo da dose recebida e da quantidade de tecido glandular incluído no campo de radiação. No entanto, a xerostomia é irreversível na maioria dos casos e, por isso, torna-se um problema grave para o paciente oncológico[22].

Os pacientes com xerostomia queixam-se de desconforto bucal, perda do paladar, dificuldades na fala, na deglutição e no uso de próteses[5]. A ausência de saliva deixa a mucosa oral com aparência seca, atrófica, pálida ou hiperêmica. A mucosa da língua pode exibir características semelhantes ou aparecer fissurada. Os lábios podem estar secos, rachados ou fissurados[5]. Diante dessas alterações, o paciente geralmente opta por alimentos macios e ricos em carboidratos[3,5].

Além disso, a saliva mantém a homeostase da cavidade oral através de várias funções como lubrificação, capacidade tampão e atividade antimicrobiana[23]. Com a diminuição do fluxo há um aumento da viscosidade e alteração dos sistemas antimicrobianos[5]. O pH salivar que normalmente é em torno de 7,0 cai para 5,0 em pacientes pós radioterapia, o que faz com que os minerais de esmalte e dentina de dissolvam com maior facilidade[7]. Além disso, há uma diminuição da capacidade remineralizante, considerando que as condições do ambiente oral de pacientes com câncer de cabeça e pescoço após o tratamento são especialmente propensas à desmineralização, associado ainda a alterações da flora oral com aumento de microrganismos acidogênicos e cariogênicos (*Streptococcus mutans*, *Lactobacillus* e *Candida*)[17].

Desse modo, fica claro que as alterações salivares são preocupantes no manejo odontológico do paciente pós radioterapia de cabeça e pescoço. Tais alterações interferem diretamente na qualidade de vida desses pacientes e na sua vida social. A hipossalivação e xerosomia faz com esses pacientes tenham dificuldade para falar, para sentir o sabor dos alimentos, para deglutir os alimentos e para dormir. Além disso a ausência de saliva contribui significativamente para o acometimento das CRR.

Alteração na composição e nas propriedades do esmalte e dentina

Diversos estudos mostram que a radioterapia altera a composição e as propriedades mecânicas dos tecidos dentários – esmalte e dentina[3, 8-15]. Os raios ionizantes utilizados na radioterapia causam a quebra da molécula de água, que corresponde a aproximadamente 4% do esmalte e 12% da dentina, gerando radicais livres de hidrogênio e peróxido de hidrogênio que são fortes reagentes de oxidação, capazes de causar a desnaturação dos componentes orgânicos das estruturas dentais[24].

O esmalte é um tecido altamente rígido e estruturalmente organizado em uma série de prismas ricos em hidroxiapatita (conteúdo mineral)[25]. A radioterapia altera principalmente a região interprismática, local onde se localiza a composição orgânica[26]. Esta região torna-se mais evidente e apresenta fissuras após a radioterapia[8]. Com a ocorrência de alterações nessa região, o esmalte torna-se menos resistente à fratura, há alteração em suas características mecânicas, microdureza e módulo de elasticidade, o que torna o tecido mais friável[26]. Além disso, detecta-se alterações na composição inorgânica, com aumento da concentração de carbonato após a radioterapia, aumentando a solubilidade do esmalte[12].

Na dentina, também são encontradas alterações químicas e mecânicas após a radioterapia. Este é um tecido mineralizado composto por 70% de matéria inorgânica (hidroxiapatita), 18% de matriz orgânica e 12% de água[25]. A matriz orgânica é composta principalmente por fibras colágenas, e são nelas que a radioterapia atua principalmente, causando sua fragmentação[8]. Alterações no colágeno faz com que ocorra uma diminuição da interação entre a matriz orgânica e os cristais de hidroxiapatita, diminuindo assim a dureza e módulo de elasticidade da dentina[11]. Há ainda alterações na porção inorgânica com troca de íons fosfato-carbonato na hidroxiapatita e diminuição da relação entre cálcio e fósforo[15], o que também torna o tecido mais solúvel em meio ácido, assim como acontece no esmalte[12,13].

Além de alterações específicas no esmalte e na dentina, uma região que se apresenta bastante instável após a radioterapia é a junção amelodentinária (JAD). A JAD é o conector natural entre esmalte duro e quebradiço e dentina subjacente, mais macia e resistente, e desempenha um papel crítico na manutenção da integridade biomecânica do dente[25]. Isso se deve ao fato de que o colágeno é afetado pela radioterapia e isso implica na instabilidade da junção[9,18]. Essa instabilidade pode causar o descolamento do esmalte, num processo chamado delaminação, com consequente exposição da dentina subjacente[9]. Clinicamente, o dente apresenta-se com aspecto quebradiço, com lascas de esmalte que podem ser removidas facilmente. O dano estrutural pode chegar a ser tão grande que causa fraturas coronárias e até mesmo radiculares[3,5,18].

Características da Cárie relacionada a radiação (CRR)

Pode-se diferenciar CRR de lesões de cárie convencionais em relação à sua localização, aparência e progressão. Quanto à localização, enquanto a cárie dentária convencional ocorre em regiões de fossas e fissuras e nas superfícies proximais entre dentes (regiões historicamente relacionadas ao acúmulo de placa bacteriana), as CRR tendem a ocorrer na região cervical (próximo à junção entre a coroa e a raiz), pontas de cúspides e bordas incisais de dentes anteriores[3]. Estes locais são expostos à grandes cargas oclusais (incisal / cúspide) e flexão associada (cervical) e são considerados resistentes à deterioração dentária causada pela cárie convencional por serem locais de mais fácil higienização. No entanto, no caso de dentes de pacientes submetidos à radioterapia, nos quais as propriedades apresentam-se alteradas e, conforme mostrado anteriormente há uma instabilidade da JAD, essas regiões se tornam menos resistentes aos esforços mastigatórios. Portanto, fatores oclusais associados às alterações mecânicas e químicas dos dentes fazem com que a CRR se desenvolva em locais comumente diferentes da cárie convencional[18]. Associado a fatores funcionais, a região da JAD é considerada um potencial local para fratura inicial do esmalte, com consequente exposição da dentina subjacente e desenvolvimento da CRR[18].

Clinicamente, três padrões da CRR podem ser observados[5]: Tipo 1 - Afeta a região cervical, padrão mais comumente visto; Tipo 2 - Aparece como áreas de desmineralização em todas as superfícies dentárias, erosões generalizadas e desgastadas superfícies oclusais e incisais são vistas; e Tipo 3 - Padrão menos comum, visto como mudanças de cor na dentina. A coroa torna-se marrom escura e pode-se observar desgastes oclusais e incisais. As figuras 1 e 2 apresentam a condição clínica de um paciente que passou por tratamento radioterápico devido à neoplasia de orofaringe.

Figura 1. Aspecto clínico de cáries relacionadas à radiação. As setas indicam diferentes características das lesões. Observa-se uma completa perda de parte do esmalte no dente 42 (delaminação) característico do padrão tipo 1. Nos demais observam-se características do padrão tipo 2 e tipo 3 com mudança de cor da dentina e aspecto de desmineralização.

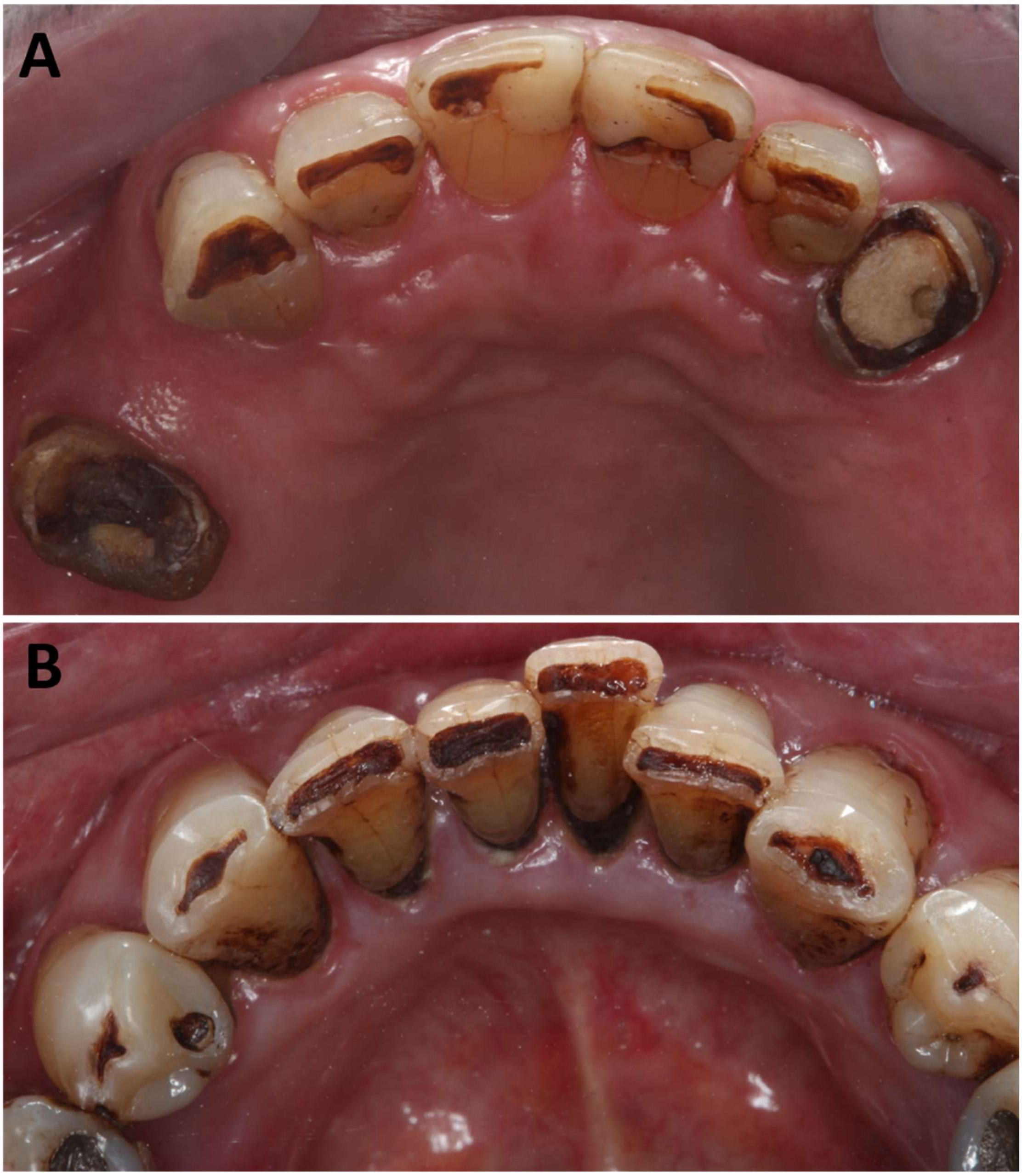

Figura 2. Visão incisal da arcada superior (A) e da arcada inferior (B). Pode-se observar os desgastes incisais e nas pontas de cúspides do pré-molares característico do padrão tipo 2. Na arcada inferior observa-se também o acometimento da região cervical lingual dos incisivos e caninos.

O local onde a CRR progride mais rapidamente é na região cervical (Figura 2B). Além das alterações mecânicas e cargas oclusais nessa região, associa-se também a ausência da proteção salivar e o acúmulo comum de placa bacteriana. Dessa forma, pode-se afirmar que uma somatória de fatores torna essa região especialmente preocupante. A CRR neste local inicia-se nas faces vestibular e lingual dos dentes, sendo mais comuns em dentes anteriores, podendo se estender por toda a região cervical[5]. Ainda na fase inicial, pode ocorrer microfissuras na região da JAD, fazendo com que haja acúmulo de bactérias neste local[18,24]. Higienização deficiente, falta de saliva e alterações intrínsecas nos tecidos dentais irradiados fazem com que a evolução da CRR seja rápida, podendo progredir para regiões mais profundas causando a amputação completa da coroa dental[5,18]. A possível perda de um elemento dentário pode acarretar muitos outros problemas. A exodontia nesses pacientes pós radioterápicos é um procedimento que requer cuidados específicos, considerando as alterações do tecido ósseo irradiado e o provável risco de osteorradionecrose[3,5,18].

Prevenção e Tratamento

É fundamental que o paciente passe pela avaliação de um cirurgião-dentista previamente à radioterapia. Dessa forma, o profissional pode orientar o paciente quanto aos cuidados e realizar os tratamentos odontológicos preventivos e curativos necessários. Restaurações de lesões cariosas e não-cariosas, trocas de restaurações insatisfatórias e qualquer outro procedimento odontológico (tratamentos endodônticos, periodontais e cirúrgicos) idealmente devem ser realizados antes da radioterapia[3]. Claro que isso dependerá de muitos fatores e nem sempre será possível.

O paciente deve ser orientado quanto à higienização e quanto à importância do uso do flúor constante a partir do início da radioterapia. Com relação à higiene diária, muitos pacientes queixam-se do creme dental. A presença de lauril sulfato de sódio, agente de formação de espuma de dentifrícios, pode remover a camada de mucina e causar úlceras na mucosa oral[22]. Outra constante queixa dos pacientes é em relação ao sabor, muitos reclamam que o sabor mentolado incomoda e contribui para causar irritação da mucosa[22]. Além disso, a abrasividade dos cremes dentais é outra preocupação. Cremes dentais com alta abrasividade não são uma boa opção, o que é indicado é a utilização de dentifrícios remineralizantes que fornecem íons solúveis de cálcio e fosfato para o ambiente oral, e funcionam como uma estratégia positiva na prevenção à CRR[27], sempre sob supervisão profissional.

Outra importante estratégia é o uso do flúor. Aplicar flúor diariamente em solução para bochechos ou em gel por meio de moldeiras são métodos efetivos para controle da cárie dentária. No entanto muitos pacientes queixam-se do uso da moldeira devido à sensibilidade da mucosa oral. Nesses casos o bochecho é mais indicado. Além da prevenção da cárie, o uso do flúor ajuda na manutenção de algumas propriedades mecânicas do esmalte, quando aplicado durante a radioterapia[12]. Assim, o paciente tem a indicação de uso de flúor durante e após a radioterapia por toda a vida como estratégia preventiva à desmineralização do esmalte.

Entretanto, nem sempre é possível impedir o aparecimento e desenvolvimento da CRR. Diante de tudo já exposto, fica claro que o tratamento restaurador da CRR é extremamente desafiador. O profissional irá trabalhar em um tecido (esmalte e dentina) alterado e, além disso, o material restaurador será sujeito a uma situação clínica bastante desfavorável. Do ponto de vista técnico, o atendimento desses pacientes também gera dificuldades e requer cuidados. Muitos pacientes desenvolvem trismo após a radioterapia, o que limita sua abertura bucal[3]. Essa limitação de abertura é a primeira dificuldade que o profissional pode encontrar. Além disso, devido à hipossalivação a mucosa oral torna-se muito sensível, o que faz com que procedimentos simples como isolamento relativo muitas vezes acabam se tornando mais complexos e tenham que ser feitos com mais cuidado[18].

Além de questões técnicas, a escolha do material restaurador mais apropriado também é complicada, sendo que idealmente o material escolhido deveria demonstrar adesão adequada, prevenir cáries secundárias e resistir à desidratação e erosão ácida[18], o que não acontece na prática clínica. Os materiais restauradores mais utilizados são cimentos de ionômero de vidro (CIV) e compósitos resinosos. Os CIV são muito utilizados em pacientes irradiados por apresentarem a vantagem de liberação de flúor local, o que contribui para a prevenção de cáries recorrentes. Eles também possuem a capacidade de se unir quimicamente à estrutura dentária. No entanto, a resistência de união de ionômero de vidro fica comprometida quando a dentina é irradiada previamente à restauração[28]. A adesão de compósitos resinosos à dentina irradiada também é comprometida quando a restauração é realizada após a radioterapia[11]. Quando os materiais são avaliados quanto às suas propriedades mecânicas e composição química, não há alteração química para nenhum dos dois frente à radiação[29]. No entanto, os CIV apresentaram maiores alterações nas propriedades mecânicas[29]. Ao avaliar clinicamente restaurações com CIV e com compósito resinoso em pacientes pós radioterapia, os CIV fornecem inibição clínica da cárie, mas se desgastam facilmente, enquanto a resina composta fornece maior integridade estrutural[30].

A figura 3 mostra um aspecto imediato após restauração com resina composta em dente irradiado e aplicação de verniz fluoretado.

Figura 3. Restauração com resina composta nos dentes 41 e 42. Nesse caso foi feito uma profilaxia e posteriormente a restauração das lesões mais avançadas. As demais lesões que apresentavam cavitação foram restauradas com cimento de ionômero de vidro como forma de adequação do meio bucal. Foi feito ainda aplicação de verniz fluoretado e orientação ao paciente sobre o uso contínuo do flúor e higienização.

Em suma, ambos os materiais apresentam vantagens e limitações. Baseado na literatura não é possível indicar nem mesmo contraindicar nenhum deles para o tratamento de CRR. Uma das principais preocupações do tratamento restaurador da CRR é a falha clínica precoce, considerando que a adesão ao susbtrato dentário irradiado (esmalte e dentina) fica comprometido após a radioterapia. Isso acontece tanto para restaurações com CIV, como para compósitos resinosos[11,28]. Assim, cabe ao cirurgião-dentista avaliar a situação clínica para determinar a melhor escolha. Em algumas situações, o CIV é utilizado em um primeiro momento como uma estratégia de adequação de meio bucal e, posteriormente, é feita a substituição da restauração pelo compósito resinoso. No entanto, sabendo das limitações da adesão ao esmalte e dentina irradiados, o acompanhamento frequente dessas restaurações é fundamental.

Considerações Finais

O manejo de pacientes com CRR se tornará cada vez mais comum na prática odontológica, uma vez que os índices de cura de neoplasias malignas têm sido maiores com os avanços da medicina atual. Cabe aos cirurgiões-dentistas se prepararem para atender com segurança este público. A presente revisão deixa claro que o tratamento da CRR é desafiador. Frente as alterações salivares e das propriedades do esmalte e da dentina, o poder de destruição dental da CRR é evidente. O ambiente bucal torna-se extremamente cariogênico, com mudanças no pH e na saliva, muitas vezes acompanhadas de uma higienização deficiente. Assim, vários são os fatores que contribuem para a rápida evolução da cárie e para o insucesso de tratamentos restauradores.

A cooperação do paciente é fator fundamental para o tratamento dessa doença. Infelizmente, muitos pacientes encontram-se desmotivados ou com dor devido à hipossalivação e/ou mucosites, o que gera dificuldades de alimentação e higienização. Somado a isto, encontra-se o fator psicológico. A aparência dos dentes após o acometimento pela CRR não é esteticamente agradável e isso pode desmotivar o paciente, fazendo com que ele não contribua com sua própria saúde bucal. Nesse sentido, é fundamental que o cirurgião-dentista atue como agente motivador deste paciente. É importante o conhecimento científico do profissional sobre os fatores específicos da CRR, para que ele possa explicar ao paciente as limitações e especificidades do tratamento. O paciente pós-radioterapia nunca poderá ter alta do consultório odontológico, pelo contrário, deverá ter retornos periódicos para acompanhamento, profilaxia, acabamento/polimento das restaurações e troca de restaurações insatisfatórias. E, mais importante ainda que os retornos, é o cuidado diário que este paciente deve ter com higienização e uso contínuo do flúor em casa, para manutenção da sua saúde bucal.

Nesse contexto, cada vez mais a prevenção e a motivação do paciente deve ser o foco da atenção dos cirurgiões-dentistas. A formação apropriada de profissionais que atuem nesta área se torna cada dia mais fundamental. Além disso, é importante que pesquisadores continuem buscando respostas que ajudem na elaboração de protocolos restauradores específicos para este tipo de pacientes, buscando, sobretudo, restabelecer a saúde e melhorar a qualidade de vida desses pacientes.

Referências

1. De Felice F, Polimeni A, Valentini V, Brugnoletti O, Cassoni A, Greco A, de Vincentiis M, Tombolini V. Radiotherapy Controversies and Prospective in Head and Neck Cancer: A Literature-Based Critical Review. Neoplasia. 2018 Mar;20(3):227-232.

2. INCA, Instituto Nacional de Câncer José Alencar Gomes da Silva. Estimativa 2018: incidência de câncer no Brasil/ Instituto Nacional de Câncer José Alencar Gomes da Silva. Rio de Janeiro: INCA, 2017, 128 p.

3. Jawad H, Hodson NA, Nixon PJ. A review of dental treatment of head and neck cancer patients, before, during and after radiotherapy: part 1. Br Dent J 2015;218(2):65-8.
4. Fregnani ER, Parahyba CJ, Morais-Faria K, Fonseca FP, Ramos PA, de Moraes FY, da Conceição Vasconcelos KG, Menegussi G, Santos-Silva AR, Brandão TB. IMRT delivers lower radiation doses to dental structures than 3DRT in head and neck cancer patients. Radiat Oncol. 2016 Sep 7;11(1):116.
5. Vissink A, Jansma J, Spijkervet FK, Burlage FR, Coppes RP. Oral sequelae of head and neck radiotherapy. Crit Rev Oral Biol Med. 2003;14(3):199-212.
6. Kielbassa AM, Hinkelbein W, Hellwig E, Meyer-Lückel H. Radiation-related damage to dentition. Lancet Oncol. 2006; 7(4):326-35.
7. Dreizen SA, Brown LR, Handler S, Levi BM. Radiation induced xerostomia in cancer patients. Cancer 1976 38:273-278
8. Gonçalves LM, Palma-Dibb RG, Paula-Silva FW, Oliveira HF, Nelson-Filho P, Silva LA, et al. Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth. J Dent. 2014;42(8):986-92.
9. Reed R, Xu C, Liu Y, Gorski JP, Wang Y, Walker MP. Radiotherapy effect on nano-mechanical properties and chemical composition of enamel and dentine. Arch Oral Biol. 2015;60(5):690-7.
10. Novais VR, Soares PB, Guimarães CM, Schliebe LR, Braga SSL, Soares CJ. Effect of gamma radiation and endodontic treatment on mechanical properties of human and bovine root dentin. Braz Dent. Journal. 2016;27(6):670-4.
11. Rodrigues RB, Soares CJ, Junior PCS, Lara VC, Arana-Chavez VE, Novais VR. Influence of radiotherapy on the dentin properties and bond strength. Clin Oral Investig. 2018; 22(2):875-883.
12. Lopes CCA, Soares CJ, Lara VC, Arana-Chavez VE, Soares PB, Novais VR. Effect of fluoride application during radiotherapy on enamel demineralization. J Appl Oral Sci. 2018; doi: 10.1590/1678-7757-2018-0044.
13. Miranda RR, Silva ACA, Dantas NO, Soares CJ, Novais VR. Chemical analysis of in vivo-irradiated dentine of head and neck cancer patients by ATR-FTIR and Raman spectroscopy. Clin Oral Investig. 2018; doi: 10.1007/s00784-018-2758-6.

14. Lu H, Zhao Q, Guo J, Zeng B, Yu X, Yu D, Zhao W. Direct radiation-induced effects on dental hard tissue. Radiat Oncol. 2019 Jan 11;14(1):5.
15. Velo MMAC, Farha ALH, da Silva Santos PS, Shiota A, Sansavino SZ, Souza AT, Honório HM, Wang L. Radiotherapy alters the composition, structural and mechanical properties of root dentin in vitro. Clin Oral Investig. 2018 Nov;22(8):2871-2878.
16. Cury, J. A. et al. Conceptualization of Dental Caries by Undergraduate Dental Students from the First to the Last Year. Braz Dent J. 2014 v. 25, n.1, p. 52-59
17. Epstein JB, Chin EA, Jacobson JJ, Rishiraj B, Le N. The relationships among fluoride, cariogenic oral flora, and salivary flow rate during radiation therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;86:286-92.
18. Gupta N, Pal M, Rawat S, Grewal MS, Garg H, Chauhan D, Ahlawat P, Tandon S,Khurana R, Pahuja AK, Mayank M, Devnani B. Radiation-induced dental caries, prevention and treatment - A systematic review. Natl J Maxillofac Surg. 2015 Jul-Dec;6(2):160-6.
19. Good JS, Harrington KJ. The hallmarks of cancer and the radiation oncologist: updating the 5Rs of radiobiology. Clin Oncol (R Coll Radiol). 2013 Oct;25(10):569-77.
20. Gookizadeh A, Emami H, Najafizadeh N, Roayaei M. Clinical evaluation of BIOXTRA in relieving signs and symptoms of dry mouth after. head and neck radiotherapy of cancer patients at Seyed-al-Shohada Hospital, Isfahan, Iran. Advanced Biomedical Research. 2012; 1 (72).
21. Wijers O, Levendag P, Braaksma M, et al.Patients with head and neck cancer cured by radiation therapy: a survey of the dry mouth syndrome in long-term survivors. Head Neck.2002; 24:737–747.
22. Dirix P, Nuyts S, Bogaert WVD. Radiation-Induced Xerostomia in Patients With Head and Neck Cancer A Literature Review. Cancer. 2006; 107 (n11):2525-34
23. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001 Feb;85(2):162-9. Review.

24. Pioch T, Golfels D, Staehle HJ. An experimental study of the stability of irradiated teeth in the region of the dentinoenamel junction. Endod Dent Traumatol. 1992;8(6):241-4.
25. NANCI, A. Ten Cate. Histologia Oral. Desenvolvimento, Estrutura e Função. 7ª ed. Elsevier Editora Ltda, 2008.
26. Santin GC, Palma-Dibb RG, Romano FL, de Oliveira HF, Nelson Filho P, de Queiroz AM. Physical and adhesive properties of dental enamel after radiotherapy and bonding of metal and ceramic brackets. Am J Orthod Dentofacial Orthop. 2015 Aug;148(2):283-92.
27. Papas A, Russell D, Singh M, Kent R, Triol C, Winston A. Caries clinical trial of a remineralising toothpaste in radiation patients. Gerodontology 2008;25:76-88.
28. Yesilyurt C, Bulucu B, Sezen O, Bulut G, Celik D. Bond strengths of two conventional glass-ionomer cements to irradiated and non-irradiated dentin. Dent Mater J. 2008 Sep;27(5):695-701
29. Brandeburski SBN, Della Bona A. Effect of ionizing radiation on properties of restorative materials. Dent Mater. 2018 Feb;34(2):221-227
30. De Moor RJ, Stassen IG, van 't Veldt Y, Torbeyns D, Hommez GM. Two-year clinical performance of glass ionomer and resin composite restorations in xerostomic head- and neck-irradiated cancer patients. Clin Oral Investig. 2011 Feb;15(1):31-8.

CONCLUSÕES

4. CONCLUSÕES

Dentro das limitações do presente trabalho pode-se concluir que:

1 – A literatura não deixa dúvidas quanto às alterações mecânicas e químicas que a radioterapia causa no esmalte e na dentina humanos.

2 – A maior parte da literatura disponível sobre adesão à esmalte e dentina irradiados mostra que o momento da realização de restaurações adesivas em esmalte e dentina é importante. Quando a restauração é realizada após a radioterapia a resistência de união é comprometida.

3- Não há padronização na literatura sobre qual o sistema adesivo ideal para restaurar esmalte e dentina irradiados.

4 – Ao avaliar um sistema adesivo convencional de três passos por meio de um estudo *in vitro* em esmalte irradiado, mostrou-se que há a formação de uma camada adesiva heterogênea quando a restauração é realizada após a radioterapia. Essa camada adesiva deficiente faz com os valores de resistência de união sejam menores quando comparados a um grupo não irradiado e a um grupo restaurado antes da radioterapia.

5 – Ao avaliar a dentina da região cervical, local mais acometido pela cárie relacionada à radiação, por meio de um estudo *in vitro,* mostrou-se que esta dentina é comprometida após a radioterapia, apresentando suas propriedades mecânicas diminuídas e alterações químicas importantes.

REFERÊNCIAS

5. REFERÊNCIAS

Caccelli EMN, Rapoport A. Para-efeito das irradiações nas neoplasias de boca e orofaringe. **Rev. Brás. Cir. Cabeça e Pescoço.** 2008**;**37(4):198-201- 208.

Conceição, EM. **Dentística - Saúde e Estética**. 2a Edição. Porto Alegre: Artes Médicas. 2007.

De Felice F, Polimeni A, Valentini V, Brugnoletti O, Cassoni A, Greco A, de Vincentiis M, Tombolini V. Radiotherapy Controversies and Prospective in Head and Neck Cancer: A Literature-Based Critical Review. **Neoplasia**. 2018 Mar;20(3):227-232. https://doi.org/10.1016/j.neo.2018.01.002

de Miranda RR, Silva ACA, Dantas NO, Soares CJ, Novais VR. Chemical analysis of in vivo-irradiated dentine of head and neck cancer patients by ATR-FTIR and Raman spectroscopy. **Clin Oral Investig**. 2018 Dec 4. doi: 10.1007/s00784-018-2758-6. https://doi.org/10.1007/s00784-018-2758-6

Fejerskov O, KIdd E. **Cárie Dentária - A doença e seu tratamento clínico**. São Paulo: Editora Santos, 2005.

Giannini M, Soares CJ, de Carvalho RM. Ultimate tensile strength of tooth structures**. Dent Mater.** 2004 May;20(4):322-9. https://doi.org/10.1016/S0109-5641(03)00110-6

Gonçalves LM, Palma-Dibb RG, Paula-Silva FW, Oliveira HF, Nelson-Filho P, Silva LA, et al. Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth. **J Dent**. 2014;42(8):986-92. https://doi.org/10.1016/j.jdent.2014.05.011

Good JS, Harrington KJ. The hallmarks of cancer and the radiation oncologist: updating the 5Rs of radiobiology. **Clin Oncol (R Coll Radiol).** 2013 Oct;25(10):569-77. https://doi.org/10.1016/j.clon.2013.06.009

* De acordo com a Norma da FOUFU, baseado nas Normas de Vancouver. Abreviaturas dos periódicos com conformidade com Medline (Pubmed)

Gupta N, Pal M, Rawat S, Grewal MS, Garg H, Chauhan D, Ahlawat P, Tandon S,Khurana R, Pahuja AK, Mayank M, Devnani B. Radiation-induced dental caries, prevention and treatment - A systematic review. **Natl J Maxillofac Surg**. 2015 Jul-Dec;6(2):160-6. https://doi.org/10.4103/0975-5950.183870

INCA, Instituto Nacional de Câncer José Alencar Gomes da Silva. **Estimativa 2018: incidência de câncer no Brasil/ Instituto Nacional de Câncer José Alencar Gomes da Silva**. Rio de Janeiro: INCA, 2017, 128 p.

INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA **Monitoramento das Ações de Controle dos Cânceres de Lábio e Cavidade Oral.** Informativo Detecção Precoce, ano 7, n. 1, 2016. Disponível em: http://www1.inca.gov.br/inca/Arquivos/informativodeteccaoprecoce_janabr2016.pdf

Jawad H, Hodson NA, Nixon PJ. A review of dental treatment of head and neck cancer patients, before, during and after radiotherapy: part 1. **Br Dent J** 2015;218(2):65-8. https://doi.org/10.1038/sj.bdj.2015.28

Jham BC, da Silva Freire AR. Oral complications of radiotherapy in the head and neck. **Braz J Otorhinolaryngol**. 2006;72(5):704-708. https://doi.org/10.1016/S1808-8694(15)31029-6

Kielbassa AM, Hinkelbein W, Hellwig E, Meyer-Luckel H. Radiation-related damage to dentition. **Lancet Oncol** 2006 7:326-335 2045(06)70658-1 https://doi.org/10.1016/S1470-2045(06)70658-1

Lazarus C, Logeman JA, Palouski BR, Radenaker AW, Helenoswski IB, Vonesh EF, et al. Effects of radiotherapy with or without chemotherapy on tongue strength and swallowing in patients oral cancer. **Head Neck,** 2007; 29: 632-637. https://doi.org/10.1002/hed.20577

Liang X, Zhang JY, Cheng IK, Li JY. Effect of high energy X-ray irradiation on the nano-mechanical properties of human enamel and dentine. **Braz Oral Res**. 2016;30. pii: S1806-83242016000100209. https://doi.org/10.1590/1807-3107BOR-2016.vol30.0009

Lieshout HFL & Bots CP. The effect of radiotherapy on dental hard tissue—a systematic review. **Clin Oral Investig** 2014;18:17–24. https://doi.org/10.1007/s00784-013-1034-z

Lopes CCA, Soares CJ, Lara VC, Arana-Chavez VE, Soares PB, Novais VR. Effect of fluoride application during radiotherapy on enamel demineralization. J **ApplOral Sci.** 2018 Dec 10;27:e20180044. https://doi.org/10.1590/1678-7757-2018-0044

Lu H, Zhao Q, Guo J, Zeng B, Yu X, Yu D, Zhao W. Direct radiation-induced effects on dental hard tissue. **Radiation oncology** 2019 14:5 doi:10.1186/s13014-019-1208-1 https://doi.org/10.1186/s13014-019-1208-1

Madrid Troconis CC, Santos-Silva AR, Brandão TB, Lopes MA, de Goes MF. Impact of head and neck radiotherapy on the mechanical behavior of composite resins and adhesive systems: A systematic review. **Dent Mater.** 2017 Nov;33(11):1229-1243. https://doi.org/10.1016/j.dental.2017.07.014

Martins, G.C. et al. Adesivos dentinários. **Rev. gaúcha Odontol**. 2008; 56(4):429-436, out./dez.

McGuire JD, Gorski JP, Dusevich V, Wang Y, Walker MP. Type IV collagen is a novel DEJ biomarker that is reduced by radiotherapy. **J Dent Res**. 2014 Oct;93(10):1028-34. https://doi.org/10.1177/0022034514548221

Nanci, A. Ten Cate. **Histologia Oral. Desenvolvimento, Estrutura e Função**. 7 ed. Rio de Janeiro: Elsevier Editora, 2008

Naves LZ, Novais VR, Armstrong SR, Correr-Sobrinho L, Soares CJ. Effect of gamma radiation on bonding to human enamel and dentin **Support Care Cancer** 2012 20:2873-2878 https://doi.org/10.1007/s00520-012-1414-y

Pioch T, Golfels D, Staehle HJ. An experimental study of the stability of irradiated teeth in the region of the dentinoenamel junction. **Endod Dent Traumatol**. 1992 Dec;8(6):241-4. https://doi.org/10.1111/j.1600-9657.1992.tb00251.x

Qing P, Huang S, Gao S, Qian L, Yu H. Effect of gamma irradiation on the wear behaviour of human tooth enamel. **Scientific reports** 2015 5:11568 https://doi.org/10.1038/srep11568

Qing P, Huang S, Gao S, Qian L, Yu H. Effect of gamma irradiation on the wear behavior of human tooth dentin **Clin Oral Investig** 2016 20:2379-2386 https://doi.org/10.1007/s00784-016-1731-5

Queiroz AM, Bonilla CMC, Palma-Dibb RG, Oliveira HF, Nelson-Filho P, Silva LAB, Lucisano MP, Paula-Silva FWG. Radiotherapy Activates and Protease Inhibitors Inactivate Matrix Metalloproteinases in the Dentinoenamel Junction of Permanent Teeth. **Caries Res**. 2018 Sep 26;53(3):253-259. https://doi.org/10.1159/000492081

Reed R, Xu C, Liu Y, Gorski JP, Wang Y, Walker MP. Radiotherapy effect on nano-mechanical properties and chemical composition of enamel and dentine. **Arch Oral Biol**. 2015 May;60(5):690-7. https://doi.org/10.1016/j.archoralbio.2015.02.020

Rodrigues RB, Soares CJ, Junior PCS, Lara VC, Arana-Chavez VE, Novais VR. Influence of radiotherapy on the dentin properties and bond strength. **Clin Oral Investig**. 2018; 22(2):875-883 https://doi.org/10.1007/s00784-017-2165-4

Santin GC, Palma-Dibb RG, Romano FL, de Oliveira HF, Nelson Filho P, de Queiroz AM. Physical and adhesive properties of dental enamel after radiotherapy and bonding of metal and ceramic brackets. **Am J Orthod Dentofacial Orthop.** 2015 Aug;148(2):283-92. https://doi.org/10.1016/j.ajodo.2015.03.025

Soares CJ, Castro CG, Neiva NA, Soares PV, Santos-Filho PCF, Naves LZ, et al. Effect of gamma irradiation on ultimate tensile strength of enamel and dentin. **J Dent Res**. 2010;89(2):159-64. https://doi.org/10.1177/0022034509351251

Velo MMAC, Farha ALH, da Silva Santos PS, Shiota A, Sansavino SZ, Souza AT, Honório HM, Wang L. Radiotherapy alters the composition, structural and mechanical properties of root dentin in vitro. **Clin Oral Investig**. 2018 Nov;22(8):2871-2878. https://doi.org/10.1007/s00784-018-2373-6

Vissink A, Burlage FR, Spijkervet FK, Jansma J, Coppes RP. Prevention and treatment of the consequences of head and neck radiotherapy. **Crit Rev Oral Biol Med.** 2003;14(3):213-225. https://doi.org/10.1177/154411130301400306

Walker MP, Wichman B, Cheng AL, Coster J, Williams KB. Impact of Radiotherapy Dose on Dentition Breakdown in Head and Neck Cancer Patients. **Pract Radiat Oncol**. 2011;1(3):142-148. https://doi.org/10.1016/j.prro.2011.03.003

Yadav S, Yadav H. Ionizing irradiation affects the microtensile resin dentin bond strength under simulated clinical conditions **J Conserv Dent** 201316:148-151 doi:10.4103/0972-0707.108198 https://doi.org/10.4103/0972-0707.108198

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O Câncer é uma doença que acomete cada vez mais a população brasileira. Quando localizado na região de cabeça e pescoço, a radioterapia é uma forma de tratamento muito utilizada, por ser capaz de matar células cancerígenas. No entanto, existe uma preocupação dos profissionais de saúde em relação aos efeitos negativos da radioterapia que podem acontecer nas regiões próximas ao tumor. Quando a radiação envolve a cavidade oral pode gerar problemas nas glândulas que produzem nossa saliva, nos dentes, na gengiva e no osso ao redor dos dentes. Dessa forma, o atendimento desses pacientes é desafiador para o cirurgião dentista. Existe a dúvida de como planejar o tratamento para este tipo de paciente já que essas alterações comprometem a longevidade do tratamento odontológico. As alterações nas glândulas salivares fazem com que diminua a quantidade de saliva presente na boca e sem ela o paciente tem dificuldade para falar, engolir e dormir. Além disso, a saliva tem função de limpeza e proteção da gengiva e dos dentes. Sua ausência faz com que diminua essa proteção natural e os dentes ficam mais susceptíveis ao aparecimento de cáries. A cárie em paciente após radioterapia é chamada de cárie relacionada à radiação e possui uma evolução muito rápida, devido a alteração que ocorre na composição dos dentes, podendo levar à sua perda. Tratar essas lesões não é fácil. O dentista precisa compreender essas alterações para explicar e orientar os seus pacientes. Os materiais rotineiramente usados no consultório para tratamento de cárie não se aderem da melhor forma ao dente que recebeu irradiação, podendo soltar da estrutura dentária com frequência, gerando um desgaste do paciente e do profissional. Pacientes com câncer de cabeça e pescoço devem colaborar por meio de cuidados de higiene oral. O uso diário de flúor deve ser feito em casa por meio de bochecho ou aplicação de gel de fluoreto de sódio a 1%, e o uso de cremes dentais remineralizantes também estão indicados, sob supervisão profissional. Neste contexto, mais estudos a respeito deste assunto são importantes e os profissionais de saúde devem sempre buscar conhecimento científico para atender esse público de maneira correta buscando a melhora da qualidade de vida. Portanto, sugere-se o acompanhamento odontológico do paciente antes, durante e após a radioterapia.

ANEXOS

Comprovante do envio do artigo – Capítulo 2

Radiation and Environmental Biophysics

Effect of radiotherapy on the bond strength of composite resin to human enamel

--Manuscript Draft--

Manuscript Number:	REBS-D-18-00208
Full Title:	Effect of radiotherapy on the bond strength of composite resin to human enamel
Article Type:	Original Article
Keywords:	Adhesion; Dental enamel; Head and Neck Neoplasms; Radiotherapy
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Abstract:	The purpose of this study was evaluating the microshear bond strength (μSBS) of composite resin to human enamel performed before or after radiotherapy (RT). Forty-five humans third molars were randomly divided into three groups (n=15): CT-