Rafael Resende de Miranda

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*Influence of different ionizing radiation protocols on dental structures and dental management of head and neck cancer patients*

Tese apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia, para obtenção do Título de Doutor em Odontologia na Área de Clínica Odontológica Integrada.

Uberlândia, 2021

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Orientadora: Prof. Dra. Veridiana Resende Novais Simamoto

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## ATA DE DEFESA - PÓS-GRADUAÇÃO

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Reuniu-se em Web Conferência pela plataforma Zoom, em conformidade com a PORTARIA Nº 36, DE 19 DE MARÇO DE 2020 da COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR - CAPES, pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Odontologia, assim composta: Professores Doutores: Gisele Rodrigues da Silva (UFU); Paulo César de Freitas Santos Filho (UFU); Ana Carolina Magalhães (USP); Francielle Silvestre Verner (UFJF); Veridiana Resende Novais (UFU) orientador(a) do(a) candidato(a).

Iniciando os trabalhos a presidente da mesa, Dra. Veridiana Resende Novais, 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 arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

[A]provado(a).

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

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e à minha irmã Viviane, por nunca medirem esforços
para que eu pudesse trilhar o caminho que escolhi, sempre
fornecendo o acolhimento e o suporte necessário para
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“A mente que se abre a uma nova ideia jamais voltará
ao seu tamanho original.”

***Albert Einstein***

# SUMÁRIO

# RESUMO

Pacientes oncológicos de cabeça e pescoço podem apresentar efeitos colaterais decorrentes do tratamento por radioterapia (RT) ou quimioterapia, incluindo complicações que impactam diretamente na área de atuação do dentista. Mucosite, xerostomia, disgeusia, trismo, disfagia, necrose de tecidos moles, osteorradionecrose e cáries relacionada à radiação são algumas das complicações orais decorrentes do tratamento oncológico. A RT é uma modalidade terapêutica amplamente utilizada no tratamento de neoplasias de cabeça e pescoço. Protocolos de RT envolvem doses diárias de 2 Gy, 5 dias por semana, num período de 5 a 7 semanas. Entretanto, não existe uma padronização no protocolo de radiação in vitro de dentes para estudos científicos, com alguns estudos aplicando a radiação ionizante de forma fracionada, enquanto outros aplicam a quantidade total de radiação em uma única dose. Dessa forma, três objetivos compõem essa tese. Objetivo 1: avaliar as propriedades químicas e mecânicas do esmalte submetido a diferentes protocolos de radiação in vitro; Objetivo 2: avaliar as propriedades químicas e mecânicas da dentina submetida a diferentes protocolos de radiação in vitro; Objetivo 3: revisar a literatura e discutir aspectos importantes no tratamento odontológico de pacientes oncológicos de cabeça e pescoço durante a pandemia de COVID-19. Nos objetivos 1 e 2, 56 terceiros molares foram divididos em sete grupos (n=8): não irradiado (NI); irradiado em dose única de 30 Gy (DU30), 50 Gy (DU50) ou 70 Gy (DU70); irradiado em doses fracionadas até 30 Gy (DF30), 50 Gy (DF50) ou 70 Gy (DF70). Após seccionamento dos dentes, os espécimes foram analisados por espectroscopia infravermelha com transformada de Fourier (FTIR), espectroscopia de energia dispersiva (EDS) e teste de microdureza Knoop (KHN). Os dados obtidos foram submetidos à análise paramétrica, com nível de significância de 5%. No objetivo 1, verificou-se que dose de 70 Gy aplicada de forma fracionada produziu alterações nas propriedades químicas e mecânicas do esmalte, sendo que a partir de 50 Gy de radiação já foram encontradas alterações na porção orgânica. Doses de 30 Gy não produziram alterações significativas no esmalte, independentemente do fracionamento ou não. No objetivo 2, a dose de 70 Gy aplicada de forma fracionada alterou a composição química e microdureza da dentina, enquanto doses de 30 Gy e 50 Gy não resultaram em alterações significativas, independente do fracionamento ou não. Portanto, conclui-se que o uso do fracionamento em estudos in vitro reproduz mais fielmente a maneira como a RT é aplicada nos pacientes oncológicos de cabeça e pescoço e que a dose de 70 Gy produz as alterações mais expressivas nos tecidos dentais. No objetivo 3, a revisão crítica discutiu o impacto da COVID-19 na saúde bucal de pacientes em tratamento antineoplásico de cabeça e pescoço, apresentando sugestões para minimizar ou controlar os efeitos colaterais. A atuação do cirurgião-dentista foi dividida em duas frentes: tratamentos preventivos para pacientes sem dor e tratamentos intervencionistas para pacientes com dor, respeitando todos os protocolos de biossegurança contra COVID-19. A decisão final do tratamento depende sempre do julgamento do profissional, levando em consideração o consentimento e a condição de saúde geral do paciente.

**PALAVRAS-CHAVE:** dentina, esmalte, radioterapia.

## ABSTRACT

Head and neck cancer patients may experience side effects resulting from radiotherapy (RT) or chemotherapy treatment, including complications that directly impact the dentist's area of expertise. Mucositis, xerostomia, dysgeusia, trismus, dysphagia, soft tissue necrosis, osteoradionecrosis and radiation-related caries are some of the oral complications resulting from cancer treatment. RT is a therapeutic modality widely used in the treatment of head and neck neoplasms. RT protocols involve daily doses of 2 Gy, 5 days a week, for a period of 5 to 7 weeks. However, there is no standardization in the protocol of in vitro radiation of teeth for scientific studies, with some studies applying ionizing radiation in a fractional form, while others apply the total amount of radiation in a single dose. Thus, three objectives are part of this thesis. Objective 1: to evaluate the chemical and mechanical properties of enamel subjected to different in vitro radiation protocols; Objective 2: to evaluate the chemical and mechanical properties of dentin subjected to different in vitro radiation protocols; Objective 3: to review the literature and discuss important aspects in the dental treatment of head and neck cancer patients during the COVID-19 pandemic. In objectives 1 and 2, 56 third molars were divided into seven groups (n=8): non-irradiated (NI); irradiated in a single dose of 30 Gy (SD30), 50 Gy (SD50) or 70 Gy (SD70); irradiated in fractionated doses up to 30 Gy (FD30), 50 Gy (FD50) or 70 Gy (FD70). After sectioning the teeth, the specimens were analyzed by Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDS) and Knoop microhardness test (KHN). The data obtained were submitted to parametric analysis, with a significance level of 5%. In objective 1, it was found that a dose of 70 Gy applied in a fractional way produced changes in the chemical and mechanical properties of the enamel, and from 50 Gy of radiation onwards, changes in the organic portion were already found. Doses of 30 Gy did not produce significant changes in enamel, regardless of fractionation or not. In objective 2, the dose of 70 Gy applied in a fractional way changed the chemical composition and microhardness of dentin, while doses of 30 Gy and 50 Gy did not result in significant changes, regardless of fractionation or not. Therefore, it is concluded that the use of fractionation in in vitro studies more faithfully reproduces the way RT is applied in head and neck cancer patients and that the dose of 70 Gy produces the most expressive changes in dental tissues. In objective 3, the critical review discussed the impact of COVID-19 on the oral health of patients undergoing head and neck antineoplastic treatment, presenting suggestions to minimize or control side effects. The performance of the dentist was divided into two fronts: preventive treatments for patients without pain and interventional treatments for patients with pain, respecting all biosafety protocols against COVID-19. The final decision on treatment always depends on the professional's judgment, taking into account the patient's consent and general health condition.

**KEYWORDS:** dentin, enamel, radiotherapy.

# 1. INTRODUÇÃO E REFERENCIAL TEÓRICO

O câncer representa atualmente um grave problema de saúde pública, figurando entre as quatro principais causas de morte prematura (antes dos 70 anos de idade) na maioria dos países (INCA, 2019). A incidência e a mortalidade por câncer vêm aumentando devido ao envelhecimento e crescimento populacional, assim como alterações no padrão de distribuição e na prevalência dos fatores de risco para o câncer, principalmente aqueles associados ao desenvolvimento socioeconômico. É observada uma mudança nos principais tipos de câncer observados nos países em desenvolvimento, com uma diminuição dos cânceres relacionados a infecções e o aumento daqueles associados à melhoria das condições socioeconômicas com a incorporação de hábitos e atitudes associados à urbanização, como sedentarismo e alimentação inadequada (Bray et al., 2018).

No Brasil, a estimativa para cada ano do triênio 2020-2022 é de 625 mil casos novos de câncer. No sexo masculino, os tipos de câncer mais frequentes serão próstata (29,2%), cólon e reto (9,1%), pulmão (7,9%), estômago (5,9%) e cavidade oral (5,0%), à exceção do câncer de pele não melanoma. Já no sexo feminino, exceto o câncer de pele não melanoma, os cânceres mais comuns serão mama (29,7%), cólon e reto (9,2%), colo do útero (7,4%), pulmão (5,6%) e tireoide (5,4%) (INCA, 2019). Dentre os tipos existentes, aqueles localizados na região de cabeça e pescoço são tumores com crescimento relativamente rápido em áreas delicadas, anatômica e funcionalmente complexas (Schutte et al., 2020). Portanto, na maior parte dos casos o tratamento se dá por meio de radioterapia (RT), aplicada de forma isolada ou em associação à cirurgia e/ou quimioterapia.

A unidade usada para mensurar a quantidade de radiação empregada durante a RT é o Gray (Gy), que informa a dose de radiação absorvida por qualquer material ou tecido humano (Kim et al., 2019). Protocolos convencionais de RT envolvem doses diárias de 2 Gy, 5 dias por semana, durante um período de 5 a 7 semanas. A dosagem cumulativa para um paciente submetido à RT de cabeça e pescoço é entre 50 e 70 Gy (Liang et al., 2016; Zhao et al., 2018). A

RT se baseia no uso de radiação ionizante que carrega energia e interage 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, levando à morte das células neoplásicas. Entretanto, além de atingir o objetivo pretendido do tratamento, altas doses de radiação ionizante na região de cabeça e pescoço resultam em várias consequências indesejáveis, que surgem durante e após a conclusão da RT e podem persistir por toda a vida do paciente. A RT pode afetar a pele, a mucosa oral, as glândulas salivares, os ossos, a dentição e todo o complexo muscular orofacial. Estima-se uma incidência de 40% de complicações orais durante a RT (Sciubba & Goldenberg, 2006; Bhandari et al., 2020). A intensidade, a progressão e a permanência subsequente dos efeitos colaterais são influenciadas pelo planejamento de RT, dose cumulativa de radiação, volume, grau de vascularização, potencial de reparo e celularidade do tecido a ser irradiado, idade do paciente e uso concomitante ou não de drogas para quimioterapia (Awwad et al., 2002; Kim et al., 2018).

As complicações decorrentes da RT têm implicações de curto e longo prazo. Com relação ao tempo decorrido desde a RT, os efeitos iniciais são visíveis durante ou imediatamente após a RT e podem permanecer de 2 a 3 semanas após o término do tratamento. Estes incluem mucosite oral, dor nos dentes e gengiva, disgeusia, trismo (devido à dor), odinofagia, disfagia, candidose e dermatite por radiação (Stenstrom & Larsson, 2011; Villavicencio et al., 2017; Bhandari et al., 2020). Efeitos colaterais agudos geralmente se resolvem com o tempo, orientação profissional e medicação. Em pacientes com dor intensa durante a RT pode ser necessário modificar o esquema de tratamento ou até mesmo interromper a RT. Como efeitos colaterais tardios, que se desenvolvem depois de meses ou anos após a conclusão da RT, pode-se citar a perda das funções das glândulas salivares, trismo (devido à fibrose muscular), osteorradionecrose e cárie relacionada a radiação (CRR) (Jawad et al., 2015; Bhandari et al., 2020).

Clinicamente, lesões de CRR se iniciam geralmente como alterações na translucidez e cor do esmalte, que tende a desenvolver pigmentação marrom ou enegrecida nas superfícies lisas dos dentes (Deng et al., 2015). A literatura afirma que essas áreas de pigmentação representam áreas microscópicas de desmineralização subsuperficial, representando áreas de cárie incipiente no esmalte não cavitado. No estágio inicial, a CRR também se apresenta com rachaduras e fissuras no esmalte (Kielbassa et al., 1997; Kielbassa et al., 1999; Kielbassa et al., 2006). As principais áreas dos dentes afetadas por CRR são as áreas cervicais (perto da junção cemento-esmalte), podendo aparecer também em bordas incisais e pontas de cúspides (Walker et al., 2011). Outra característica peculiar da CRR é o fato que ela comumente se desenvolve nas superfícies linguais dos dentes anteriores inferiores, que geralmente não são locais comuns para cáries convencionais (Deng et al., 2015; Palmier et al., 2017).

Quando a CRR progride, a delaminação do esmalte tende a ocorrer, expondo a dentina subjacente a um ambiente oral altamente cariogênico e favorecendo a progressão rápida e agressiva da destruição dentária (Santos-Silva et al., 2015). À medida que avança a destruição da estrutura dentária ao redor da região cervical, ocorre uma diminuição do suporte da coroa dentária que eventualmente fratura, causando a amputação da coroa, deixando a raiz exposta ao meio bucal (Kielbassa et al., 2006; Madrid et al., 2017; Palmier et al., 2017). O aumento da incidência dessas lesões pode ser explicado pela associação de fatores diretos e indiretos relacionados à RT, como hipossalivação, pH ácido, dieta cariogênica, dificuldade de higienização, assim como mudanças nas propriedades biomecânicas do esmalte e da dentina irradiados (Jawad et al., 2015; Bhandari et al., 2020).

A literatura fornece evidências de alterações morfológicas, químicas e mecânicas dos tecidos dentais submetidos à radiação ionizante (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Qing et al., 2016; Lopes et al., 2018; Rodrigues et al., 2018; Velo et al., 2018; Campi et al., 2019; Lu et al., 2019; Miranda et al., 2019). Estudos relatam que dentes irradiados sofrem alterações significativas como redução da resistência à tração e compressão e diminuição da microdureza (Gonçalves et al., 2014; Reed et al., 2015). Esses fatores podem levar à desestabilização da junção amelodentinária (JAD) e aumento de metaloproteinases (MMPs-20) na matriz da junção, o que, por sua vez, pode levar à degradação dos componentes proteicos da JAD, consequentemente gerando trincas e delaminação do esmalte (Gonçalves et al., 2014; Reed et al., 2015; Fonseca et al., 2020).

Estudos *in vitro* também encontraram padrões desorganizados de dentina intertubular e peritubular submetidas à radiação ionizante, estando isso correlacionado com alterações na microdureza da dentina, favorecendo a propagação de fissuras no esmalte e interferindo na adesão de materiais restauradores resinosos (Naves et al., 2012; Yadav & Yadav, 2013; Rodrigues et al., 2018). Quimicamente, há uma redução na cristalinidade juntamente com redução no conteúdo mineral e proteico, tanto no esmalte quanto na dentina (Reed et al., 2015; Lu et al., 2019; Miranda et al., 2019). A degeneração dos componentes orgânicos e minerais também enfraquece a interação entre os cristais de hidroxiapatita, levando a um enfraquecimento da estrutura e maior solubilidade em pH baixo (Lu et al., 2019). A literatura sugere ainda que a RT leva à destruição do tecido conjuntivo e alterações morfológicas nos processos odontoblásticos, influenciando a resposta da polpa ao dano cariogênico. Além disso, estudos têm demonstrado que a RT pode causar inflamação e isquemia de forma dose-dependente, diminuindo temporariamente a resposta pulpar aos testes sensoriais (Kataoka et al., 2011; Kataoka et al., 2016).

Entretanto, metodologicamente não existe uma padronização no protocolo de radiação *in vitro* de amostras dentais para realização de estudos científicos. Alguns estudos aplicaram a radiação ionizante de forma fracionada (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Qing et al., 2016; Lopes et al., 2018; Rodrigues et al., 2018; Campi et al., 2019; Lu et al., 2019), simulando a forma como a RT é aplicada nos pacientes oncológicos, enquanto outros aplicaram a quantidade total de radiação em uma única dose (Pioch et al., 1992; da Cunha et al., 2016; da Cunha et al., 2017; Velo et al., 2018), reduzindo tempo e custo do experimento. Contudo, as diferenças no processo de irradiação podem resultar em achados conflituosos na literatura (Lieshout & Bots, 2014). Alguns trabalhos encontraram alterações nas propriedades do esmalte e da dentina irradiados (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Qing et al., 2016; Rodrigues et al., 2018; Campi et al., 2019; Lu et al., 2019), enquanto outros não (Galetti et al., 2014; da Cunha et al., 2016; da Cunha et al., 2017). A padronização nesse procedimento de irradiação *in vitro* poderia contribuir para o entendimento mais aprofundado das alterações intrínsecas dos tecidos dentais frente à RT, assim como para o delineamento de futuros estudos clínicos.

Portanto, a RT pode predispor os pacientes a sequelas de difícil controle e tratamento. As consequências inevitáveis da RT por si só limitam a interação social e a rotina dos sobreviventes oncológicos de cabeça e pescoço, diminuindo drasticamente sua qualidade de vida (Jawad et al., 2015; Bhandari et al., 2020). Conscientização, prevenção e manejo com relação a essas consequências são essenciais para os profissionais de odontologia envolvidos nos cuidados bucais desses sobreviventes. O conhecimento aliado às pesquisas científicas nessa área é importante para facilitar a formulação de estratégias preventivas, planejamento de tratamento e obtenção de saúde bucal neste grupo de pacientes (Bhandari et al., 2020). Dessa forma, o objetivo desta tese é investigar a influência da radiação ionizante sobre os tecidos dentais duros, simulando diferentes protocolos de radiação *in vitro*, bem como revisar a literatura e discutir aspectos importantes no tratamento odontológico de pacientes oncológicos de cabeça e pescoço.

## 2. CAPÍTULO 1

### Artigo 1:

Miranda RR, Ribeiro TE, Silva ELCD, Simamoto Júnior PC, Soares CJ, Novais VR. Effects of fractionation and ionizing radiation dose on the chemical composition and microhardness of enamel. *Arch Oral Biol*. 2021 Jan;121:104959. doi: 10.1016/j.archoralbio.2020.104959.

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# Effects of fractionation and ionizing radiation dose on the chemical composition and microhardness of enamel

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ABSTRACT

*Objective:* To evaluate the chemical and mechanical properties of enamel submitted to different in vitro radiation protocols.

*Design:* Third molars were divided into seven groups ($n = 8$): non-irradiated (NI); a single dose of 30 Gy (SD30), 50 Gy (SD50), or 70 Gy (SD70) of radiation; or fractional radiation doses of up to 30 Gy (FD30), 50 Gy (FD50), or 70 Gy (FD70). Hemisections were analysed by Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDS) and Knoop microhardness (KHN) test. One-way ANOVA followed by Bonferroni's post-hoc test compared the test groups with the NI. Two-way ANOVA was performed for the fractionation and radiation dose, followed by Bonferroni's test ($\alpha = 0.05$).

*Results:* FTIR revealed differences for the amide I band between the NI and FD50 and NI and FD70 groups ($p < 0.001$). For the organic matrix/mineral ratio, the FD70 group presented a lower ratio compared to NI ($p = 0.009$). Excluding the NI group, there were differences between the FD30 and FD50 ($p = 0.045$) and the FD30 and FD70 groups ($p < 0.001$). For EDS, there were differences for Ca ($p = 0.011$) and Ca/P ($p < 0.001$), with the FD70 group presenting lower values compared to NI ($p = 0.015$; $p < 0.001$). For KHN, the FD70 group presented lower values than the NI ($p = 0.002$). Two-way ANOVA showed difference for the dose ($p < 0.001$), with the 70 Gy group presenting a lower KHN value within the fractionated groups.

*Conclusion:* Fractional doses 70 Gy irradiation caused chemical and mechanical changes to enamel. Radiation applied in single or fractional doses produced different effects to enamel.

## 1. Introduction

Head and neck cancers (HNCs) comprise a heterogeneous group of neoplasms originating in the oral cavity, oropharynx, hypopharynx and larynx. Depending on the stage and site of the cancer, radiotherapy (RT) is one of the most important phases of treatment (Schutte et al., 2020). It can be used as the primary therapy, as an adjuvant to surgery and chemotherapy, or as a palliative treatment for inoperable cases and more advanced stages of HNC (Buglione et al., 2016). Despite being a highly effective non-invasive treatment for cancer control, RT causes many adverse reactions that significantly affect the patient's quality of life (Buglione et al., 2016; Villa & Akintoye, 2018). Adverse effects in the oral cavity include mucositis, dysphagia, dysgeusia, candidiasis, trismus, xerostomia, osteoradionecrosis, and radiation-related caries (Bhandari, Soni, Bahl, & Ghoshal, 2020; Jawad, Hodson, & Nixon, 2015; Villa & Akintoye, 2018).

To plan RT treatment, radiation oncologists use the gray (Gy) unit, which is the international unit of measurement of absorbed ionizing radiation and is defined as the absorption of 1 J of radiation by 1 kg of matter (Kim, Walters, & Patel., 2019). Most treatment plans for HNC patients consist of doses ranging from 50 to 70 Gy, divided into daily fractions of 2 Gy, 5 days per week over a period of 5 to 7 weeks (Liang,

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Zhang, Cheng, & Li, 2016; Zhao, Maquilan, Jiang, & Schwartz, 2018). However, there is no standardization in the process of in vitro irradiation of dental samples for scientific studies. Some studies apply ionizing radiation in fractional daily doses, simulating the way that the radiation is clinically applied in cancer patients (Arid et al., 2020; Campi et al., 2019; Gonçalves et al., 2014; Lopes, Soares et al., 2018; Lu et al., 2019; Novais et al., 2016; Qing, Huang, Gao, Qian, & Yu, 2015; Qing, Huang, Gao, Qian, & Yu, 2016; Reed et al., 2015; Rodrigues et al., 2018), while others apply the total dosage of radiation in a single dose (Pioch, Golfels, & Staehle, 1992; da Cunha, Ramos et al., 2016, 2017; Velo et al., 2018).

These differences in the irradiation process result in conflicting findings in the literature. Studies using a single dosage found no differences in the bond strength of adhesive systems to dental substrates (da Cunha, Ramos et al., 2016) or in the micromorphology of irradiated teeth (da Cunha, Fonseca et al., 2017), while other studies using fractional doses found chemical and mechanical changes in enamel and dentin (Campi et al., 2019; Gonçalves et al., 2014; Lu et al., 2019; Novais et al., 2016; Qing et al., 2015, 2016; Reed et al., 2015; Rodrigues et al., 2018). Focusing on enamel, ionizing radiation seems to cause changes in its chemical composition, microhardness, elastic modulus and wear behaviour, making the enamel more susceptible to demineralization (Lopes, Soares et al., 2018; Qing et al., 2015; Reed et al., 2015; Seyedmahmoued et al., 2018).

Thus, there are no homogeneous responses about the direct damage caused by ionizing radiation to teeth in experimental studies since different protocols and radiation doses are used in vitro. It is necessary to simulate these variations in techniques and dosages to understand at what level changes in dental tissues occur when submitted to RT. Therefore, the aim of this study was to evaluate the chemical and mechanical changes in human enamel submitted to different radiation doses, applied in single or fractionated doses. The null hypothesis tested was that different protocols and radiation doses do not alter the chemical composition and microhardness of enamel.

## 2. Materials and Methods

### 2.1. Irradiation and sample preparation

Fifty-six non-carious human third molars were collected, cleaned, and stored in deionized water at a temperature of 4 °C for up to 3 months after extraction (Ferreira et al., 2016; Lopes, Soares et al., 2018; Miranda, Silva, Dantas, Soares, & Novais, 2019). This study was approved by the Ethical Committee in Research of the Federal University of Uberlândia (Protocol 2.910.276). Teeth were fixed in utility wax plates (NewWax, Technew, Rio de Janeiro, Brazil) and submitted to different RT protocols using a linear accelerator (Clinac 600C Varian®, Palo Alto, CA, USA; beam 6 MV). After sample calculation, the teeth were randomly divided into seven experimental groups according to the irradiation protocol ($n = 8$): non-irradiated (NI); a single dose of 30 Gy (SD30), 50 Gy (SD50), or 70 Gy (SD70); or fractional doses up to 30 Gy (FD30), 50 Gy (FD50), or 70 Gy (FD70). Single-dose groups received single-session irradiation according to the total dose established for each group. Fractionated groups were exposed to 2 Gy fractions, 5 days a week, until doses equal to 30, 50 or 70 Gy were reached. Samples were submerged in deionized water during the irradiation process. The NI group was stored in deionized water at 4 °C until the other groups completed irradiation treatment (Lopes, Soares et al., 2018).

For sample preparation, teeth were cut at the cementoenamel junction using a water-cooled diamond saw (Isomet, 15HC diamond; Buehler Ltd., Lake Bluff, IL, USA) mounted on a precision saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA). Each crown was sectioned in the mesio-distal direction, resulting in two halves: buccal and lingual. These halves were again sectioned buccolingually to generate four hemisections. The mesiobuccal hemisections were analysed by attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FTIR) and energy dispersive X-ray spectroscopy (EDS). The distobuccal hemisections were submitted to the Knoop microhardness (KHN) test.

### 2.2. ATR/FTIR

The chemical composition of the samples was determined using ATR/FTIR (Vertex 70, Bruker, Ettlingen, Germany). The ATR/FTIR unit contained a deuterated triglycine sulfate (DTGS) detector. After manual planning, the flat surfaces were positioned against the diamond crystal of the ATR/FTIR unit and pressed with a force gauge at a constant pressure to facilitate contact. Spectra were recorded in the range of 400 to 4,000 $cm^{-1}$ at 4 $cm^{-1}$ resolution. Each specimen was scanned 32 times, and the final spectrum acquired was the average of these scans. Spectra were recorded and analysed by OPUS 6.5 software (Bruker, Ettlingen, DEU).

After baseline correction and normalization, the area under each band was integrated by using the appropriate tools from the software. Each spectrum was normalized according to the phosphate band (1,190 - 702 $cm^{-1}$). FTIR spectra were analysed by calculating the following parameters: (1) organic matrix/mineral ratio (M:M), expressed as the ratio between the integrated areas of protein amide I (1,655 $cm^{-1}$) and the phosphate $v_1$, $v_3$ stretching mode (960 and 1,040 $cm^{-1}$); and (2) relative carbonate content (RCC), expressed as the ratio of the integrated areas of the two strongest carbonate $v_3$ peaks at 1,460 and 1,425 $cm^{-1}$ and the phosphate $v_1$, $v_3$ stretching mode (Fig. 1). In enamel, M:M represents the amount of organic matrix relative to the inorganic matrix, and RCC evaluates the extent of carbonate incorporated into hydroxyapatite (Lopes, Limirio, Novais, & Dechichi, 2018).

### 2.3. EDS

The distribution of mineral components and the elemental composition were analysed using energy dispersive X-ray spectroscopy (EDS). The specimens were assembled into stubs, sputter-coated with gold (Au) and observed using a scanning electron microscope (LEO-1430; Carl Zeiss, BW, Oberkochen, Germany). The regions of interest were amplified 1,000×, and the composition and distribution of elements were analysed by EDS (Oxford Instruments, England). The concentrations by weight of the following chemical elements were evaluated: Ca (calcium), O (oxygen), P (phosphorus) and C (carbon). From the data obtained, the ratios of calcium to phosphorus (Ca/P) and carbon to phosphorus (C/P) were also calculated.

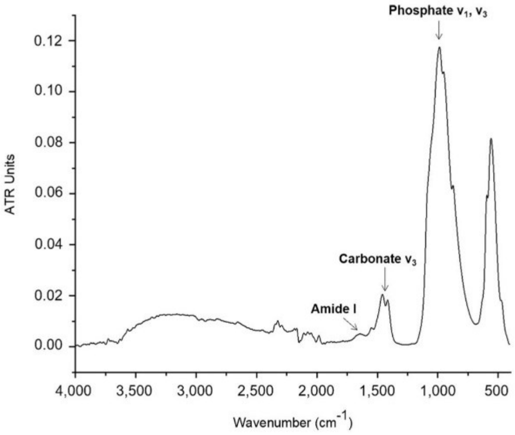

**Fig. 1.** ATR/FTIR spectrum for non-irradiated enamel. The amide I (1,655 $cm^{-1}$), carbonate $v_3$ (1,460 and 1,425 $cm^{-1}$) and phosphate $v_1$, $v_3$ (960 and 1,040 $cm^{-1}$) bands were analysed.

### 2.4. Microhardness test

To evaluate the Knoop microhardness (KHN) of the enamel, the specimens were embedded in polystyrene resin (AM 190 resin; Aerojet, São Paulo, SP, Brazil). The surfaces were ground with silicon carbide paper grit sizes #600, 800, 1200 and 2000 (Norton, Campinas, SP, Brazil) and polished with felt discs and metallographic diamond pastes (6, 3, 1, and ¼ μm grit; Arotec, São Paulo, SP, Brazil) (Novais et al., 2016; Rodrigues et al., 2018). The entire process on the polishing machine was timed and done by a single operator. The Knoop indentation values were determined with a micro durometer (FM 700; FutureTech Corp., Kawasaki, Japan) by applying a load of 50 g for 10 s (Torres, Zanatta, Silva, & Borges, 2019). Indentations were performed parallel to the direction of the enamel prisms. Five indentations with a distance of 100 μm between them were made into each specimen, and these measurements were later averaged to determine the KHN of the specimens.

### 2.5. Statistical analysis

Data from all methods were tested for normal distribution (Shapiro-Wilk's test, $\alpha = 0.05$) and equality of variances (Levene's test, $\alpha = 0.05$), followed by parametric statistical tests. To compare the test groups with the NI group, data were analysed using one-way ANOVA followed by Bonferroni's post-hoc test. Excluding the NI group, two-way ANOVA was performed for the study factors (fractionation and radiation dose), followed by Bonferroni's post-hoc test. Sigma Plot statistical package (version 12.0, Systat Software, Inc., San Jose, CA, USA) was used for analysis, and a p value of less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. ATR/FTIR

The mean and standard deviations of the integrated area of each chemical component analysed are presented in Table 1, as well as the M:M ratio and RCC. For chemical components, one-way ANOVA showed statistical significance for amide I ($p < 0.001$) and phosphate ($p < 0.001$). Regarding amide I, multiple comparisons with Bonferroni correction revealed differences between the NI and FD50 groups and between the NI and FD70 groups ($p < 0.001$ for both). For phosphate, there was a significant difference only between the NI and FD70 groups ($p < 0.001$). There was no significant difference regarding carbonate ($p = 0.086$). For the ratios, one-way ANOVA showed statistical significance for M:M ($p < 0.001$) and RCC ($p = 0.019$). The M:M ratio was significantly lower in the FD70 group than in the NI group ($p = 0.009$). On the other hand, Bonferroni's correction found no difference between the NI group and each irradiated group for the RCC ($p > 0.05$).

**Table 1**
Means ± the standard deviations of the integrated area of each chemical component; M:M and RCC by ATR/FTIR spectroscopy.

| Group | Amide I | Phosphate | Carbonate | M:M | RCC |
|---|---|---|---|---|---|
| NI | 0.32 ± 0.16 | 11.54 ± 0.56 | 1.76 ± 0.27 | 0.020 ± 0.011 | 0.16 ± 0.02 |
| SD30 | 0.29 ± 0.07 | 11.91 ± 0.40 | 1.97 ± 0.47 | 0.020 ± 0.006 | 0.18 ± 0.04 |
| SD50 | 0.20 ± 0.06 | 11.32 ± 0.62 | 1.74 ± 0.39 | 0.016 ± 0.005 | 0.15 ± 0.03 |
| SD70 | 0.24 ± 0.08 | 11.15 ± 0.46 | 1.53 ± 0.40 | 0.025 ± 0.011 | 0.16 ± 0.04 |
| FD30 | 0.23 ± 0.06 | 11.25 ± 0.43 | 1.72 ± 0.26 | 0.020 ± 0.005 | 0.17 ± 0.02 |
| FD50 | 0.15 ± 0.03* | 11.40 ± 0.28 | 1.59 ± 0.30 | 0.013 ± 0.004 | 0.14 ± 0.03 |
| FD70 | 0.11 ± 0.03* | 10.89 ± 0.50 * | 1.44 ± 0.13 | 0.010 ± 0.003* | 0.13 ± 0.01 |

[*] Indicates a significant difference compared to the NI group ($p < 0.05$).

Excluding the NI group, two-way ANOVA revealed statistical significance for M:M in relation to the fractionation factor ($p < 0.001$), the dose factor ($p = 0.029$) and the interaction between them ($p = 0.002$). Within the fractionated groups, Bonferroni's correction revealed differences between the 30 and 50 Gy doses ($p = 0.045$) and between the 30 and 70 Gy doses ($p < 0.001$), with the 50 and 70 Gy groups showing lower values for M:M. Regarding the fractionation or not of ionizing radiation, there was a significant difference only between the 70 Gy groups ($p < 0.001$), and the fractionated dose group presented a lower M:M ratio than the single dose group. For RCC, two-way ANOVA found a significant difference for the dose factor ($p = 0.008$) but not for the fractionation factor ($p = 0.083$) or for the interaction between them ($p = 0.452$). Bonferroni's correction revealed statistical significance between the 30 and 50 Gy doses ($p = 0.017$) and between the 30 and 70 Gy doses ($p = 0.026$), as seen in Table 2.

### 3.2. EDS

The mean and standard deviations for the concentrations of each chemical element are presented in Fig. 2, as well as the Ca/P and C/P ratios. One-way ANOVA showed statistical significance only for Ca ($p = 0.011$) and Ca/P ($p < 0.001$). Bonferroni's correction revealed lower Ca and Ca/P values for FD70 ($p = 0.015$ and $p < 0.001$, respectively) compared to the NI group. There were no significant differences for C ($p = 0.078$), O ($p = 0.468$), P ($p = 0.455$) or C/P ($p = 0.222$).

Excluding the NI group, two-way ANOVA revealed statistical significance for Ca/P in relation to the fractionation factor ($p < 0.001$), the dose factor ($p < 0.001$) and the interaction between them ($p < 0.001$). Within the fractionated groups, Bonferroni's correction revealed differences between the 30 and 70 Gy doses ($p < 0.001$) and between the 50 and 70 Gy doses ($p < 0.001$), with the 70 Gy group showing a lower Ca/P ratio. Regarding fractionation, there was a significant difference only between the 70 Gy groups ($p < 0.001$), and the fractionated dose group presented a lower Ca/P ratio than the single dose group. For the C/P ratio, two-way ANOVA found no significant difference for the fractionation factor ($p = 0.133$), dose factor ($p = 0.289$), or for the interaction between them ($p = 0.765$), as shown in Table 3.

### 3.3. Knoop microhardness

The means and standard deviations for the KHN values are shown in Table 4. One-way ANOVA showed a significant difference between the groups ($p = 0.001$). Bonferroni's correction revealed a significant difference between the NI and FD70 groups ($p = 0.002$), with the latter presenting a lower KHN value.

Excluding the NI group and performing a two-way ANOVA, there was a statistical significance only for the dose factor ($p < 0.001$). No significance was found for the isolated fractionation factor ($p = 0.156$) or for the interaction between fractionation and dose ($p = 0.295$). Within the fractionated groups, Bonferroni's correction showed a lower

**Table 2**
Means ± the standard deviations for M:M and RCC by ATR/FTIR spectroscopy, comparing fractionation and radiation dose.

| | M:M | | RCC | |
|---|---|---|---|---|
| Radiation dose | Single | Fractionated | Single | Fractionated |
| 30 Gy | 0.020 ± 0.006 Aa | 0.020 ± 0.005 Aa | 0.18 ± 0.04 Aa | 0.17 ± 0.02 Aa |
| 50 Gy | 0.016 ± 0.005 Aa | 0.013 ± 0.004 Ba | 0.15 ± 0.03 Ba | 0.14 ± 0.03 Ba |
| 70 Gy | 0.025 ± 0.011 Aa | 0.010 ± 0.003 Bb | 0.16 ± 0.04 Ba | 0.13 ± 0.01 Ba |

Different uppercase letters (analysis in columns) and lowercase letters (analysis in rows) represent significant differences ($p < 0.05$).

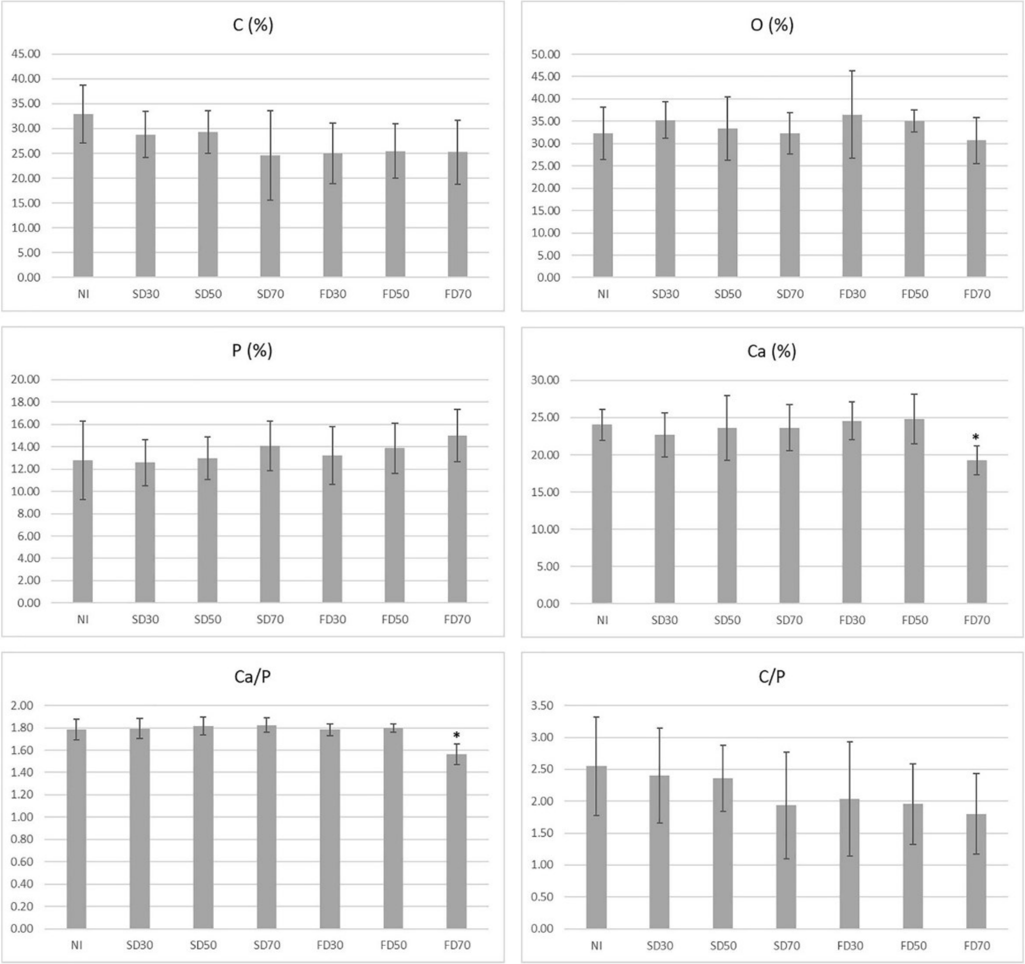

**Fig. 2.** Means and standard deviations for the concentrations of each chemical element and Ca/P and C/P ratios analysed by EDS. Footnote: *Indicates significant difference compared to the NI group ($p < 0.05$).

**Table 3**
Means ± the standard deviations for the Ca/P and C/P ratios, comparing fractionation and radiation dose.

| | Ca/P ratio | | C/P ratio | |
|---|---|---|---|---|
| Radiation dose | Single | Fractionated | Single | Fractionated |
| 30 Gy | 1.79 ± 0.09<br>Aa | 1.78 ± 0.05<br>Aa | 2.40 ± 0.75<br>Aa | 2.03 ± 0.90<br>Aa |
| 50 Gy | 1.82 ± 0.08<br>Aa | 1.80 ± 0.04<br>Aa | 2.36 ± 0.52<br>Aa | 1.95 ± 0.63<br>Aa |
| 70 Gy | 1.82 ± 0.07<br>Aa | 1.56 ± 0.09<br>Bb | 1.93 ± 0.84<br>Aa | 1.80 ± 0.63<br>Aa |

Different uppercase letters (analysis in columns) and lowercase letters (analysis in rows) represent significant differences ($p < 0.05$).

**Table 4**
Means ± standard deviations for the KHN values of the enamel including the NI group.

| Group | Mean (±) | p value |
|---|---|---|
| NI | 342.47 ± 29.99 | - |
| SD30 | 347.64 ± 35.01 | p = 1.000 |
| SD50 | 333.76 ± 21.68 | p = 1.000 |
| SD70 | 313.45 ± 33.87 | p = 0.255 |
| FD30 | 333.30 ± 19.91 | p = 1.000 |
| FD50 | 338.96 ± 26.62 | p = 1.000 |
| FD70 | 288.22 ± 24.28* | p = 0.002 |

[*] Indicates a significant difference compared to the NI group ($p < 0.05$).

KHN value for the 70 Gy group that was significantly different from the 30 Gy ($p = 0.006$) and 50 Gy ($p = 0.002$) groups.

## 4. Discussion

The null hypothesis was rejected since different protocols and radiation doses alter the chemical composition and microhardness of enamel. This probably occurred due to the interaction of ionizing radiation with the chemical components of the dental structure, with the radiation's ability to change the molecular conformation of the dental structure, consequently resulting in mechanical changes (Fang, Yang, & Wu, 2002; Fränzel, Gerlach, Hein, & Schaller, 2006).

Enamel is characterized as the most mineralized tissue in the human body. It consists of 96% inorganic matrix in the form of apatite crystals and the remainder is an organic matrix (3.5% water and 0.5% protein material) (Teruel, Alcolea, Hernández, & Ruiz, 2015). HNC patients present post-radiation dental lesions that start as enamel fractures, which may result in complete delamination of this substrate, suggesting instability of the enamel/dentin junction (Jansma, Vissink, Jongebloed, Retief, & Johannes's-Gravenmade, 1993; Lieshout & Bots, 2014; Pioch et al., 1992). In this sense, it is important to study the chemical composition of enamel and its changes when subjected to ionizing radiation. ATR/FTIR spectroscopy provides information about the chemical composition of the samples by molecular vibrations (Cui et al., 2012; Lopes, Limirio et al., 2018). The advantages of this technique include obtaining a good signal-to-noise ratio, achieving high-quality spectra in a short time and the possibility of analysing materials in their natural form without the need for specific preparations (Lopes, Limirio et al., 2018).

In ATR/FTIR, the M:M ratio evaluates the amount of organic matrix relative to the inorganic matrix (Lopes, Soares et al., 2018). The organic matrix influences the quantity, quality and distribution of mineral crystals and may change the hierarchical architecture of enamel and affect its mechanical properties (Sa et al., 2014). In the present study, changes in the organic matrix were verified by an evaluation of amide I, which corresponds to the most intense absorption band in proteins (Miranda et al., 2019; Xu & Wang, 2012). The FD70 group showed a difference in the M:M ratio when compared to the NI group, while the FD50 and FD70 groups showed lower values for amide I. These findings are corroborated by previous studies that have demonstrated that high doses of ionizing radiation alter the organic matrix of dental tissues (Arid et al., 2020; Gonçalves et al., 2014; McGuire et al., 2014; Miranda et al., 2019; Rodrigues et al., 2018; Soares et al., 2010). In turn, RCC indicates the carbonate content of the samples. Carbonate is the impurity that gives enamel greater solubility and instability and represents 3% of enamel dry weight (Xu, Reed, Gorski, Wang, & Walker, 2012). Compared to the NI group, the different radiation protocols did not change the RCC values, probably because the samples were not stored in an acidic pH medium or subjected to demineralization protocols capable of causing carbonate dissolution, making the carbonate more soluble or changing its quantity (Lopes, Soares et al., 2018; Xu et al., 2012). In the clinical reality of HNC patients, these factors are present during and after radiation, mainly due to a preference for cariogenic foods, changes in saliva and difficulties in oral hygiene (Bhandari et al., 2020; Jawad et al., 2015).

Regarding the dose factor, the doses of 50 and 70 Gy showed lower M:M ratio values compared to the dose of 30 Gy dose within the fractionated groups. For the RCC, the 50 and 70 Gy groups showed lower values of this chemical parameter than the 30 Gy groups. After 50 Gy of irradiation, changes in the chemical composition can be noticed, especially with regard to the organic part. These results are consistent with the findings of Walker, Wichman, Cheng, Coster, and Williams (2011); Reed et al. (2015) and Lu et al. (2019), since the increase in the radiation dose was directly proportional to the chemical changes found in the enamel. Notably, in most patients, RT protocols for HNC are based on pre-established patterns in fractional doses of 2 Gy, which may vary from 50 to 70 Gy depending on the state of tumour development (Liang et al., 2016; Zhao et al., 2018). In the most severe cases, it is recommended to apply daily therapeutic doses until reaching values equal to or greater than 70 Gy to obtain favourable results regarding tumour regression (Bhandari et al., 2020; Rades, Janssen, Bajrovic, Strojan, & Schild, 2016). However, it is important to consider that, depending on the stage and location of the HNC, the amount of radiation that reaches the dental structures varies considerably (Fregnani et al., 2016).

EDS spectroscopy is a specific method for determining the concentration of chemical elements on the substratum (Cakir, Korkmaz, Firat, Oztas, & Gurgan, 2011). It relies on the application of high-energy electrons on the samples and observation of X-ray emissions resulting from the core-hole via the de-excitation of electrons. The X-ray energy emitted will generally differ from element to element considering that each element has a unique atomic structure (Mitić et al., 2017). The elements detected by EDS in the present study are among the main forming elements of the hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] (Kudkuli, Abdulla, Rekha, Sharma, & Gurjar, 2019). Overall, the FD70 group showed lower Ca and Ca/P values compared to the NI group. Within the fractionated groups, the dose of 70 Gy showed a lower of Ca/P value compared to the doses of 30 and 50 Gy. The proportional decrease in these values with increasing radiation dose indicated variation in the inorganic content of the enamel (Kudkuli et al., 2019). Since there are 10 Ca ions per unit of hydroxyapatite, calcium activity increases the solubility equation to the tenth power (Simmer & Fincham, 1995), and its solubility is directly related to enamel strength during pH cycling. Enamel strength is more affected by changes in Ca concentration than by changes in any other factor in the tooth structure and the external environment (Ferreira et al., 2016). The Ca/P ratio is considered an indicator of mineralization degree (Gerth, Dammaschke, Schäfer, & Züchner, 2007), and a decrease in this content results in decreased mechanical properties (Xu et al., 2012). Therefore, a decrease in the Ca and Ca/P values in teeth submitted to high doses of ionizing radiation (70 Gy) can trigger the appearance of radiation-related caries in HNC patients.

Analysing the mechanical properties, the FD70 group was the only group with a lower microhardness value than the NI group. Regarding the dose factor, the dose of 70 Gy caused a decrease in KHN value only within the fractionated groups. The current results are similar to previous studies that reported a decrease in the elastic modulus and hardness after high doses of radiation (Fränzel & Gerlach, 2009; Fränzel et al., 2006; Lieshout & Bots, 2014; Lu et al., 2019; Qing et al., 2015; Reed et al., 2015). This decrease in microhardness is probably due to post-irradiation reduction of inorganic content. It is worth remembering that there is a high interaction between ionizing radiation and water present in the tissues (Cole & Silver, 1963). Despite being present in a small amount in the enamel, radiolysis occurs and free radicals are released that can interact with other ions to produce new compounds. This would explain the decrease in Ca ions and the lower Ca/P ratio after irradiation (Velo et al., 2018). In addition, impairment of the interprismatic region weakens the connection between apatites, which can lead to microcracks and a rough enamel surface (Knychalska-Karwan, Pawlicki, & Karwan, 1988). On the other hand, a study conducted by Liang et al. (2016) found changes in the mechanical properties of hard dental tissues even at doses of 10-30 Gy, which was not found in the present study. In view of all these methodologies, the FD70 group showed the lowest microhardness value and concomitantly the amide I, M:M ratio, Ca content and Ca/P ratio values. Then, the chemical and mechanical changes found in this group may justify greater susceptibility to enamel caries in patients undergoing head and neck RT, since the protocols recommended in oncological treatment use fractional doses of 50 to 70 Gy. Based on the present results, the fractional doses up to 70 Gy clearly contributes to the breakdown of the chemical structure and a reduction in surface microhardness.

The changes found in the enamel subjected to ionizing radiation can be explained by the decarboxylation of the tissue. The organic matrix interacts with the apatite crystals through calcium ions from electrostatic binding of collagen side chains carboxylate and surface mineral phosphate groups. As collagen is composed of macromolecular chains of various types of amino acids, irradiation can cause side chain decarboxylation and the loss of acidic phosphate groups. The mineral-organic interaction between apatite and collagen is reduced and may induce microcracks in the hydroxyapatite mineral. The decarboxylation-related changes on a molecular level may affect the mineral content, composition, structure, and, subsequently, the mechanical properties of the enamel (Fränzel & Gerlach, 2009; Hübner, Blume, Pushnjakova, Dekhtyar, & Hein, 2005; Liang et al., 2016).

In the present study, the application of radiation by single or fractional doses caused different changes in the enamel. The fractional dosage groups showed changes in both the chemical and mechanical properties of the enamel, while the single dose irradiation groups did not. Although the application of a single dose facilitates the execution of in vitro studies, by reducing the time of the experiment and the use of the equipment, it does not reproduce the way in which RT is delivered to HNC patients. The effects of RT are cumulative, and fractionation of radiation is based on the "5Rs" (repair, redistribution, reoxygenation, regeneration and radiosensitivity) (Harrington, Jankowska, & Hingorani, 2007). Despite knowing that none of the “5Rs” could occur in teeth in an in vitro study, a fractionated daily dose of 2 Gy was used to simulate clinical conditions and more accurately reproduce what is done during head and neck RT (Arid et al., 2020). Thus, the direct consequences of ionizing radiation on dental structures could be better evaluated.

Oral health guidelines are extremely necessary for HNC patients. Maintaining adequate oral hygiene is essential, using a soft toothbrush and dental floss or an interproximal brush (Buglione et al., 2016). Since the fluoride ion has the ability to bond with apatite to form fluorhydroxyapatite, the use of fluoride is a great preventive measure to promote remineralization and inhibit demineralization of dental surfaces. Thus, daily use of fluoride mouthwashes (with or without chlorhexidine) and a neutral topical sodium fluoride gel is recommended during and after RT to protect the teeth from damage (Buglione et al., 2016; Lopes, Soares et al., 2018; Soares et al., 2011).

However, our study has some limitations. First, the irradiation protocol used has been simplified and does not reproduce aspects such as pH and xerostomia present in the oral cavities of patients. Second, bonding tests were not performed to verify the influence of fractionation and radiation dose on the adhesion of restorative materials. Besides that, the use of young teeth whose composition is different from aged teeth in cancer patients who need RT (Gupta, Johnson, & Kumar, 2016) can be considered another limitation, since significant changes in organic matrices and mineral density occur with age (Carvalho & Lussi, 2017). To control the variability in this in vitro study, the type of tooth and the region analysed were standardized (Hobson, McCabe, & Hogg, 2001), as well as the age of the tooth donors (Reed et al., 2015). Despite this, both chemical and mechanical changes in enamel were detected when high doses of ionizing radiation were applied. Clinical studies are needed to confirm the results of this in vitro study. Additional clinical studies are necessary to create protocols with the purpose of minimizing or counteracting direct radiation damage to dental tissues.

## 5. Conclusions

Under the conditions of this in vitro study, 70 Gy of radiation delivered in fractional doses was able to produce changes in both the chemical and mechanical properties of the enamel. From 50 Gy of fractionated radiation, alterations can be found mainly in relation to organic components. Doses of 30 Gy did not produce significant changes in the enamel, regardless of the use or not of fractionation. Ionizing radiation applied in single or fractional doses produced different effects on the evaluated properties; however, the use of fractionation more faithfully reproduces the way in which RT is applied to HNC patients.

## Authors’ contributions

Rafael R. de Miranda: conception and design of the study, acquisition of data and manuscript drafting. Thalles E. Ribeiro: preparation of specimens, acquisition of data and manuscript drafting. Edna L. C. da Silva: acquisition of data and manuscript review. Paulo C. Simamoto Júnior: data analysis and manuscript review. Carlos J. Soares: data analysis and manuscript review. Veridiana R. Novais: conception and design of the study and manuscript review.

## Declaration of Competing Interest

The authors report no declarations of interest.

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# 3. CAPÍTULO 2

## Artigo 2:

## Effects of fractionation and ionizing radiation dose on the chemical composition and microhardness of dentin

Artigo a ser submetido para publicação no periódico Archives of Oral Biology.

# Effects of fractionation and ionizing radiation dose on the chemical composition and microhardness of dentin

Running title: Characterization of irradiated dentin

## Abstract

*Objective:* To analyse the chemical and mechanical properties of dentin submitted to different in vitro radiation protocols.

*Design:* Fifty-six third molars were divided into seven groups (n=8): non-irradiated (NI); a single radiation dose of 30 Gy (SD30), 50 Gy (SD50), or 70 Gy (SD70); or fractional radiation doses of up to 30 Gy (FD30), 50 Gy (FD50), or 70 Gy (FD70). Dentin hemisections were evaluated by Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDS) and Knoop microhardness (KHN). One-way ANOVA followed by Dunnett's test compared the test groups with the NI. Two-way ANOVA was used to compare fractionation and radiation dose, followed by Tukey's test (α=0.05).

*Results:* FTIR showed differences for carbonate values between the NI and FD70 groups (p=0.005), as well as for the C:M (p<0.001) and amide I/amide III ratio (p=0.014). In EDS, there was a difference only for the Ca content (p=0.014), with the FD70 group presenting lower values compared to the NI. For KHN, the FD70 group had lower values than the NI (p<0.001). Two-way ANOVA revealed differences for the interaction between fractionation and dose (p=0.005), with the 70 Gy fractionated group presenting lower microhardness values.

*Conclusions:* Fractional doses 70 Gy irradiation resulted in changes in both the chemical and mechanical properties of the dentin. Doses of 30 Gy and 50 Gy did not result in significant changes to dentin, regardless of fractionation. Radiation applied in single doses did not produce any change in dentin, regardless of dose.

Keywords: Chemical composition, Dentin, Fourier transform infrared spectroscopy, Hardness, Radiotherapy.

## 1. Introduction

Radiotherapy (RT) plays a key role in the treatment of head and neck cancer (HNC) as a primary and sole treatment or in combination with surgery and chemotherapy (Bhandari et al., 2020). The choice of the therapeutic modality is based on tumour location, clinical staging, histological grade of malignancy, tumor volume, healthy structures present in the region and the patient's physical condition (Schutte et al., 2020). Ionizing radiation acts on deoxyribonucleic acid (DNA) causing cell death or loss of its reproductive capacity and, despite advances in radiation techniques, patients still have side reactions that can compromise cancer treatment and quality of life (Szturz et al., 2017; Bhandari et al., 2020). In this sense, mucositis, dermatitis, xerostomia, dysphagia, dysgeusia, trismus, osteoradionecrosis and radiation-related caries are the most common sequelae observed in patients undergoing RT in the head and neck region, which may manifest as acute or late effects (Bhandari et al., 2020; Rinstad et al., 2020).

The application of RT occurs using a controlled radiation dose directed to the tumour mass and the variable used to measure the absorbed radiation dose is the gray (Gy). The treatment is used by the fractional method, delivering 1.8 Gy and 2 Gy daily, 5 days a week, for 5 to 7 weeks. Thus, the final dosage that the patient receives of ionizing radiation is between 50 Gy and 70 Gy, depending on the oncologist's planning (Liang et al., 2016; Zhao et al., 2018). In addition, the literature reports that doses of 50 Gy or higher cause critical changes in the chemical and mechanical properties of dental hard tissues (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Lopes et al., 2018; Rodrigues et al., 2018; Lu et al., 2019; Miranda et al., 2019), making them more susceptible to caries.

To understand these changes and propose ways to inhibit or delay them, in vitro dental radiation models are often used in research. Some studies apply ionizing radiation in the same way as it is done in HNC patients, with daily divided doses (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Qing et al., 2016; Lopes et al., 2018; Rodrigues et al., 2018; Campi et al., 2019; Lu et al., 2019; Arid et al., 2020), while others apply radiation in a single dose, justifying that post-extraction teeth do not have vital cells (Pioch, Golfels, & Staehle, 1992; da Cunha et al., 2016; da Cunha et al., 2017; Velo et al., 2018). Therefore, there is no standardization in the way in which in vitro radiation is used in dental samples and this can generate conflicting results in the literature (Lieshout & Bots, 2014). Thus, some research found alterations in irradiated enamel and dentin (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Novais et al., 2016; Qing et al., 2016; Rodrigues et al., 2018; Campi et al., 2019; Lu et al., 2019; Miranda et al., 2021), while others did not (Galetti et al., 2014; da Cunha et al., 2016; da Cunha et al., 2017).

Therefore, simulating these variations in techniques and dosages is necessary to understand the intrinsic changes that occur in dental substrates concerning to RT (Miranda et al., 2021). Due to its higher water content, dentin seems to be more vulnerable to the effects of ionizing radiation, since RT affects water molecules forming free radicals (Pioch, Golfels, & Staehle, 1992; Gonçalves et al., 2014). This study, therefore, aimed to evaluate the chemical and mechanical changes in human dentin when subjected to different in vitro radiation protocols. The null hypothesis tested was that different protocols and radiation doses do not alter the chemical composition and microhardness of dentin.

## 2. Materials and Methods

### *2.1. Irradiation and specimen preparation*

After approval by the Ethical Committee in Research of the Federal University of Uberlândia (Protocol 2.910.276), 56 non-carious human third molars were collected. The teeth were cleaned with periodontal curettes and pumice prophylaxis (SSWhite, Petrópolis, RJ, Brazil) with a Robson brush (KG Sorensen, SP, Brazil) in a low-speed handpiece. The teeth were stored in deionized water at a temperature of 4ºC for up to 3 months after extraction. For in vitro irradiation, the teeth were fixed in utility wax plates (NewWax, Technew, Rio de Janeiro, Brazil) and submitted to different RT protocols using a linear accelerator (Clinac 600C Varian®, Palo Alto, CA, USA; beam 6 MV). Teeth were randomly divided into seven groups according to the irradiation protocol (n=8): non-irradiated (NI); a single dose of 30 Gy (SD30), 50 Gy (SD50), or 70 Gy (SD70); or fractional doses up to 30 Gy (FD30), 50 Gy (FD50), or 70 Gy (FD70). The single-dose groups received ionizing radiation in a single session according to the total dose established for each group. The fractionated groups were exposed to 2 Gy fractions, 5 days a week, until they reached a total dose of 30, 50 or 70 Gy. Teeth were submerged in deionized water during the irradiation process. The process of RT was conducted by an experienced and trained physician. The NI group was stored in deionized water at 4°C until the other groups completed the irradiation (Miranda et al., 2021).

After irradiation, teeth were cut at the cementoenamel junction using a water-cooled diamond saw (Isomet, 15HC diamond; Buehler Ltd., Lake Bluff, IL, USA) mounted on a precision saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA) for root removal. Then, a new cut was made 3 mm above towards the occlusal surface, obtaining a 3 mm thick slice. After removing the enamel, each slice was sectioned in the mesio-distal direction, obtaining two halves: buccal and lingual. These halves were again sectioned in the buccolingual direction, obtaining four hemisections. The mesiobuccal hemisections were used for analysis by attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FTIR) and energy dispersive X-ray spectroscopy (EDS). The distobuccal hemisections were submitted to the Knoop microhardness (KHN) test.

### *2.2. ATR/FTIR*

Chemical composition was determined for each group using attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FTIR; Vertex 70, Bruker, Ettlingen, Germany). The ATR/FTIR unit contained a deuterated triglycine sulfate (DTGS) detector. The testing surfaces were positioned against the diamond crystal of the ATR/FTIR unit and pressed with a force gauge at a constant pressure to facilitate contact. The spectra were recorded in the range from 400 to 4,000 $cm^{-1}$ at a 4 $cm^{-1}$ resolution. The specimens were scanned 32 times in each FTIR measurement, and the spectrum acquired is the average of all these scans. Spectra were recorded and analysed by OPUS 6.5 software (Bruker, Ettlingen, Germany).

Each spectrum was normalized according to the phosphate band. After baseline correction and normalization, the FTIR spectra were analysed by calculating the following parameters: (1) mineral/matrix ratio (M:M): the band ratio between 1,035 and 1,655 $cm^{-1}$, attributed to the $v_1$, $v_3$ vibration of phosphate ion and the C=O stretching of collagen amide I, respectively; (2) carbonate/mineral ratio (C:M): the ratio of the integrated areas of carbonate $v_2$ at 872 $cm^{-1}$ to the phosphate $v_1$, $v_3$ at 1,035 $cm^{-1}$; (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 ratio of the integrated areas of amide I at 1,655 $cm^{-1}$ to the $CH_2$ scissoring at 1,450 $cm^{-1}$ (Rodrigues et al., 2018; Miranda et al., 2019) (Fig. 1).

### *2.3. EDS*

The distribution of chemical elements in dentin samples was analysed using energy dispersive X-ray spectroscopy (EDS). The specimens were assembled into stubs, sputter-coated with gold (Au) and observed under a scanning electron microscope (LEO-1430; Carl Zeiss, BW, Oberkochen, Germany). The regions of interest were amplified 1,000×, and the composition and distribution of the elements were analysed by EDS (Oxford Instruments, England) with an accelerating voltage of 20 kV. The concentrations by weight of the following elements were evaluated: Ca (calcium), O (oxygen), P (phosphorus) and C (carbon). From the data obtained, the ratios of calcium to phosphorus (Ca/P) and carbon to phosphorus (C/P) were also calculated (Kudkuli et al., 2019).

### *2.4. Microhardness test*

To evaluate Knoop microhardness (KHN), dentin specimens were embedded in polystyrene resin (AM 190 resin; Aerojet, São Paulo, SP, Brazil). The surfaces were ground with silicon carbide paper grit sizes #600, 800, 1200 and 2000 (Norton, Campinas, SP, Brazil) for 60 seconds each. Then they were polished with felt discs and metallographic diamond pastes (6, 3, 1, and ¼ µm grit; Arotec, São Paulo, SP, Brazil) in a polishing machine. Knoop indentation values were obtained using a microdurometer (FM 700; FutureTech Corp., Kawasaki, Japan) by applying a 25 g load for 5 s (Marangoni-Lopes et al., 2019). Indentations were performed perpendicularly to the direction of the dentinal tubules. Five indentations were made per specimen and a distance of 100 μm between them was recommended. These measurements were averaged to determine the KHN of each specimen and subsequently for each group.

### *2.5. Statistical analysis*

Data were tested for normal distribution (Shapiro-Wilk's test, $\alpha=0.05$) and equality of variances (Levene's test, $\alpha=0.05$), followed by parametric tests. To compare the test groups with the NI group, data were analysed using one-way ANOVA followed by Dunnett's test. Excluding the NI group, two-way ANOVA was performed for the study factors (fractionation and radiation dose), followed by Tukey's post hoc test. Sigma Plot statistical package (version 12.0, Systat Software, Inc., San Jose, CA, USA) was used and a p value of less than 0.05 was considered statistically significant.

## 3. Results

### *3.1. ATR/FTIR*

The mean and standard deviations of the integrated area of each chemical component and the ratios obtained in all groups are shown in Table 1. For chemical components, one-way ANOVA showed no statistical difference for amide I ($p=0.153$), phosphate ($p=0.140$), $CH_2$ ($p=0.833$) and amide III ($p=0.162$). There was a significant difference only for carbonate ($p=0.005$). In relation to the carbonate values, Dunnett's test revealed a difference between the NI and FD70 groups ($p=0.002$). For the ratios, one-way ANOVA showed statistical significance for C:M ($p<0.001$) and amide I/amide III ($p=0.014$). The C:M ratio was significantly higher in the FD70 group compared to the NI ($p=0.002$), while the amide I/amide III ratio was significantly lower in the FD70 group compared to the NI ($p=0.002$).

There were no differences for the M:M (p=0.170) and amide I/$CH_2$ (p=0.243) ratios.

Excluding the NI group, two-way ANOVA revealed statistical significance for C:M in relation to the dose factor (p<0.001) and the interaction between fractionation and dose (p=0.015). Within the fractionated groups, Tukey’s test revealed differences between the doses of 30 and 70 Gy (p<0.001) and 50 and 70 Gy (p<0.001), with 70 Gy group presenting higher values for C:M. Two-way ANOVA revealed statistical significance for amide I/amide III in relation to the fractionation factor (p=0.018), but not for the dose factor (p=0.615) and for the interaction between them (p=0.073). Regarding fractionation, Tukey’s test showed a significant difference only between the 70 Gy groups (p=0.002), with the fractionated dose group presented a lower amide I/amide III ratio than the single dose group. Two-way ANOVA showed no statistical significance for M:M (fractionation p=0.187; dose p=0.226; interaction p=0.995) and neither for amide I/$CH_2$ (fractionation p=0.128; dose p=0.752; interaction p=0.148), as seen in Table 2.

**Table 1.** Means ± the standard deviations of the integrated area of each chemical component and the ratios evaluated by ATR/FTIR spectroscopy for all groups.

| Group | Amide I | Phosphate | Carbonate | $CH_2$ | Amide III | M:M | C:M | Amide I/ amide III | Amide I/ $CH_2$ |
|---|---|---|---|---|---|---|---|---|---|
| NI | 2.10 ± 0.25 | 16.48 ± 0.45 | 0.23 ± 0.02 | 0.25 ± 0.04 | 0.22 ± 0.03 | 8.18 ± 1.02 | 0.015 ± 0.001 | 13.24 ± 1.45 | 9.60 ± 1.55 |
| SD30 | 2.35 ± 0.44 | 15.86 ± 0.84 | 0.24 ± 0.02 | 0.27 ± 0.04 | 0.23 ± 0.04 | 7.57 ± 1.31 | 0.016 ± 0.001 | 12.06 ± 1.86 | 8.31 ± 1.85 |
| SD50 | 2.30 ± 0.33 | 16.30 ± 0.38 | 0.25 ± 0.04 | 0.26 ± 0.06 | 0.21 ± 0.03 | 7.79 ± 0.93 | 0.016 ± 0.002 | 12.09 ± 2.29 | 8.33 ± 1.67 |
| SD70 | 2.33 ± 0.56 | 15.80 ± 0.32 | 0.26 ± 0.04 | 0.27 ± 0.05 | 0.21 ± 0.02 | 7.10 ± 1.33 | 0.017 ± 0.002 | 12.93 ± 2.19 | 8.98 ± 1.85 |
| FD30 | 2.60 ± 0.35 | 16.53 ± 0.87 | 0.24 ± 0.03 | 0.25 ± 0.02 | 0.24 ± 0.05 | 7.09 ± 1.11 | 0.015 ± 0.002 | 11.54 ± 2.18 | 9.93 ± 1.90 |
| FD50 | 2.57 ± 0.36 | 16.46 ± 0.72 | 0.24 ± 0.02 | 0.25 ± 0.01 | 0.23 ± 0.04 | 7.38 ± 1.28 | 0.015 ± 0.002 | 11.69 ± 1.83 | 9.63 ± 1.50 |
| FD70 | 2.23 ± 0.37 | 16.34 ± 0.68 | 0.30 ± 0.05* | 0.26 ± 0.04 | 0.25 ± 0.03 | 6.67 ± 0.79 | 0.019 ± 0.002* | 9.54 ± 1.74* | 8.36 ± 1.43 |

*Indicates a significant difference compared to the NI group at Dunnett's test ($p<0.05$).

**Table 2.** Means ± the standard deviations of the ratios evaluated by ATR/FTIR spectroscopy comparing fractionation and radiation dose.

| Radiation dose | M:M | | C:M | | Amide I/amide III | | Amide I/$CH_2$ | |
|---|---|---|---|---|---|---|---|---|
| | Single | Fractionated | Single | Fractionated | Single | Fractionated | Single | Fractionated |
| 30 Gy | 7.57 ± 1.31 Aa | 7.09 ± 1.11 Aa | 0.016 ± 0.001 Aa | 0.015 ± 0.002 Aa | 12.06 ± 1.86 Aa | 11.54 ± 2.18 Aa | 8.31 ± 1.85 Aa | 9.93 ± 1.90 Aa |
| 50 Gy | 7.79 ± 0.93 Aa | 7.38 ± 1.28 Aa | 0.017 ± 0.002 Aa | 0.015 ± 0.002 Aa | 12.09 ± 2.29 Aa | 11.69 ± 1.83 Aa | 8.33 ± 1.67 Aa | 9.63 ± 1.50 Aa |
| 70 Gy | 7.10 ± 1.33 Aa | 6.67 ± 0.79 Aa | 0.017 ± 0.002 Aa | 0.019 ± 0.002 Ba | 12.93 ± 2.19 Aa | 9.54 ± 1.74 Ab | 8.98 ± 1.85 Aa | 8.36 ± 1.43 Aa |

Different uppercase letters for comparing radiation dose (analysis in columns) and lowercase letters for comparing fractionation (analysis in rows) represent significant differences at Tukey's test ($p<0.05$).

### *3.2. EDS*

The mean and standard deviations for the concentrations of chemical elements are presented in Fig. 2, as well as the Ca/P and C/P ratios, comparing each irradiated group with the NI. One-way ANOVA showed statistical significance only for Ca ($p=0.014$). Dunnett's test revealed lower Ca values for FD70 compared to the NI group ($p=0.004$). There were no significant differences for C ($p=0.633$), O ($p=0.911$), P ($p=0.890$), Ca/P ($p=0.897$) or C/P ($p=0.932$).

Excluding the NI group, two-way ANOVA revealed no significant difference for Ca/P in relation to the fractionation factor ($p=0.969$), dose factor ($p=0.446$), or the interaction between them ($p=0.617$). For the C/P ratio, two-way ANOVA found no significant difference for fractionation ($p=0.756$), dose ($p=0.479$), or the interaction between them ($p=0.986$), as shown in Table 3.

**Table 3.** Means ± the standard deviations for the Ca/P and C/P ratios comparing fractionation and radiation dose.

| **Radiation dose** | **Ca/P ratio** | | **C/P ratio** | |
|---|---|---|---|---|
| | Single | Fractionated | Single | Fractionated |
| 30 Gy | 1.82 ± 0.03 Aa | 1.84 ± 0.03 Aa | 2.90 ± 0.83 Aa | 2.97 ± 0.85 Aa |
| 50 Gy | 1.82 ± 0.03 Aa | 1.81 ± 0.04 Aa | 2.73 ± 0.71 Aa | 2.85 ± 0.66 Aa |
| 70 Gy | 1.83 ± 0.04 Aa | 1.82 ± 0.04 Aa | 2.60 ± 0.76 Aa | 2.62 ± 0.72 Aa |

Different uppercase letters for comparing radiation dose (analysis in columns) and lowercase letters for comparing fractionation (analysis in rows) represent significant differences at Tukey's test ($p<0.05$).

### *3.3. Knoop microhardness*

The means and standard deviations for the KHN values for all groups are shown in Table 4. One-way ANOVA revealed a significant difference between the groups ($p<0.001$). Dunnett's test showed a difference between the NI and FD70 groups ($p<0.001$), with the latter presenting lower KHN values.

Excluding the NI group, two-way ANOVA revealed statistical significance for the interaction between fractionation and dose (p=0.005). Within the fractionated groups, Tukey's test revealed differences between the doses of 30 and 70 Gy (p=0.014) and 50 and 70 Gy (p<0.001), with 70 Gy group presenting lower KHN values. Regarding fractionation, there was a significant difference only between the 70 Gy groups (p<0.001), with the fractionated group showing lower KHN values than the single dose group, as seen in Table 5.

**Table 4.** Means ± standard deviations for the KHN values of the dentin for all groups.

| **Group** | **Mean (±)** | **p value** |
|---|---|---|
| NI | 64.68 ± 2.85 | - |
| SD30 | 61.77 ± 4.71 | p=0.451 |
| SD50 | 60.43 ± 4.19 | p=0.127 |
| SD70 | 62.34 ± 3.03 | p=0.662 |
| FD30 | 61.32 ± 2.57 | p=0.312 |
| FD50 | 63.02 ± 3.21 | p=0.888 |
| FD70 | 55.53 ± 5.18* | p<0.001 |

*Indicates a significant difference compared to the NI group at Dunnett's test (p<0.05).

**Table 5.** Means ± standard deviations for the KHN values of the dentin comparing fractionation and radiation dose.

| **Radiation dose** | **Fractionation** | |
|---|---|---|
| | Single | Fractionated |
| 30 Gy | 61.77 ± 4.71 Aa | 61.32 ± 2.57 Aa |
| 50 Gy | 60.43 ± 4.19 Aa | 63.02 ± 3.21 Aa |
| 70 Gy | 62.34 ± 3.03 Aa | 55.53 ± 5.18 Bb |

Different uppercase letters for comparing radiation dose (analysis in columns) and lowercase letters for comparing fractionation (analysis in rows) represent significant differences at Tukey's test (p<0.05).

## 4. Discussion

The null hypothesis was rejected since different protocols and radiation doses changed the chemical composition and microhardness of dentin. Dentin has high water content and the interaction between ionizing radiation and water is known to be high (Pioch, Golfels, & Staehle, 1992; Gonçalves et al., 2014). Thus, when radiolysis occurs, $H^+$ and $OH^-$ are released and then can interact with other ions to produce chemical changes in the tissue (Velo et al., 2018), which can impact the mechanical properties as well.

Dentin is a mineralized tissue that has the function of supporting the enamel, compensating for its friable characteristic, which is why it has a greater organic content in its composition. Among the components, the most present in the organic part is collagen, being type I the most abundant (Xu & Wang, 2012). Collagen is responsible for the greater resilience of dentin in relation to enamel and its inorganic portion is mainly composed of hydroxyapatite (Scheffel et al., 2020). In the ATR/FTR analysis, the different radiation protocols were not able to change the M:M ratio, which represents the amount of organic matrix in relation to the inorganic. However, it showed a significant difference of the FD70 group compared to the NI for carbonate values and C:M ratio, showing the interference of ionizing radiation in the inorganic components of dentin. The increase in carbonate values after high radiation doses makes the tissue more susceptible to acids, causing imbalance and accelerating the process of dissolution of the tooth structure (Tartari et al., 2016; Campi et al., 2019).

Regarding to the organic portion, the FD70 group showed changes in the amide I/amide III ratio, both compared to the NI and SD70 groups. This ratio concerns the organization of collagen in dentin (Toledano et al., 2015), showing the ability of radiation to promote changes in the collagenous matrix of this dental tissue. This altered collagen may account for the rapid progression of radiation-related caries in post-RT patients (Jawad, Hodson, & Nixon, 2015; Bhandari et al., 2020). This type of caries occurs predominantly in the cervical region and can extend and lead to coronary amputation (Walker et al., 2011; Bhandari et al., 2020). In addition, with collagen impairment, there may be poor adhesion between restoration and dentinal substrate of teeth located in the radiation field, since the altered structure makes it difficult to create the hybrid layer and, consequently, makes it impossible to carry out long-lasting restorations (Yadav & Yadav, 2013; Gonçalves et al., 2014; Rodrigues et al., 2018).

The different radiation protocols did not detect changes in the concentrations of carbon, oxygen, and phosphorus. However, the FD70 group showed lower calcium values compared to the NI. These elements obtained by EDS are among the main elements that constitute hydroxyapatite crystals (Kudkuli et al., 2019; Miranda et al., 2021). In addition to $Ca_{10}(PO_4)_6(OH)_2$, there is a considerable amount of calcium-deficient apatite, which is referred to as $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(-OH)_{2-x}$. This kind of calcium-deficient apatite is less stable than $Ca_{10}(PO_4)_6(OH)_2$. When demineralization occurs, calcium-deficient apatite is easily dissolved, leading to reduced crystallinity. Consequently, irradiated dentin would be more vulnerable to acid attack than dentin with intact hydroxyapatite (al-Nawas et al., 2000; Lu et al., 2019). Therefore, the use of fluoride products is considered beneficial for the prevention of radiation-related caries in post-RT patients (Soares et al., 2011; Lopes et al., 2018).

Regarding mechanical properties, the present study showed lower dentin microhardness values in the FD70 group, which was irradiated with a high dose of radiation. These findings are corroborated by the results of previous studies (Lieshout & Bots, 2014; Gonçalves et al., 2014; Velo et al., 2018; Marangoni-Lopes et al., 2019). As it is a biological and complex tissue, dentin hardness is dependent on several factors, including the location of penetration and the local composition of the tissue (Ryou et al., 2012), hence the need to standardize the region of analysis in all specimens. Furthermore, the mechanical properties of dentin vary according to the mineral content (Zhang et al., 2014), that is, low hardness values can be explained by changes in carbonate, calcium and C:M ratio values in the present study.

Previous studies have reported that radiation doses have a greater effect on teeth as doses are increased (Gonçalves et al., 2014; de Siqueira Mellara et al., 2014). In the present study, significant changes were only detected when the accumulated dose was 70 Gy. This finding is contrasted by other studies that found degenerative changes in dental hard tissues after exposure to 30 Gy of radiation or more (Gonçalves et al., 2014; Liang et al., 2016; Lu et al., 2019). However, some of them used different irradiation protocols, with different radiation sources and with direct exposure of dentin specimens to irradiation, without the protection of enamel or periodontal tissues.

According to the literature, some studies used single-dose irradiation as a methodology (da Cunha et al., 2016; da Cunha et al., 2017; Velo et al., 2018), while others used fractional doses of radiation (Gonçalves et al., 2014; Qing et al., 2015; Reed et al., 2015; Novais et al., 2016; Qing et al., 2016; Rodrigues et al., 2018; Campi et al., 2019; Lu et al. , 2019), generating divergent results. The effects of RT are cumulative, and fractionation of radiation is based on the "5Rs" (repair, redistribution, reoxygenation, regeneration and radiosensitivity) (Harrington, Jankowska, & Hingorani, 2007). According to the results of this study, the use of fractional radiation for in vitro studies is recommended, seeking to more faithfully simulate how RT is clinically administered to HNC patients (Jawad, Hodson, & Nixon, 2015). Thus, the chances of bias in scientific studies would be smaller.

However, the present study has as a limitation the fact that it was not possible to simulate the attenuation effect of the jaw bones and oral soft tissues to radiation, nor reduced salivary flow, common characteristics in HNC patients. Even so, chemical and mechanical changes were found in dentin when high doses of ionizing radiation were applied fractionally. Therefore, it is important that further studies are carried out to discover methods that minimize these direct effects of radiation on tooth structure. Thus, the prevention and treatment of RT side effects are priority issues for future research in the area, due to the increasing survival rates of HNC patients.

## 5. Conclusions

A radiation dose of 70 Gy delivered in fractional doses resulted in changes in both the chemical and mechanical properties of the dentin. Doses of 30 Gy and 50 Gy did not result in significant changes in the dentin, regardless of fractionation or not. The use of fractional radiation for in vitro studies more faithfully reproduces the way in which RT is applied to HNC patients.

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# Figures

**Fig. 1** ATR/FTIR spectrum for non-irradiated dentin. The carbonate $v_2$ (872 cm$^{-1}$), phosphate $v_1$, $v_3$ (1,035 cm$^{-1}$), amide III (1,235 cm$^{-1}$), $CH_2$ (1,450 cm$^{-1}$) and amide I (1,655 cm$^{-1}$) bands were evaluated.

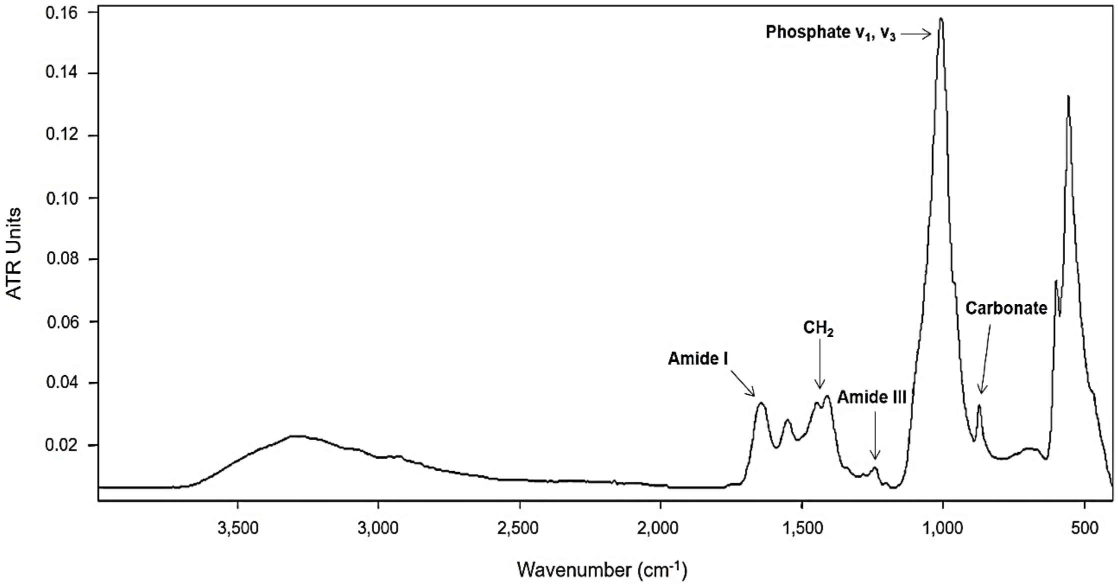
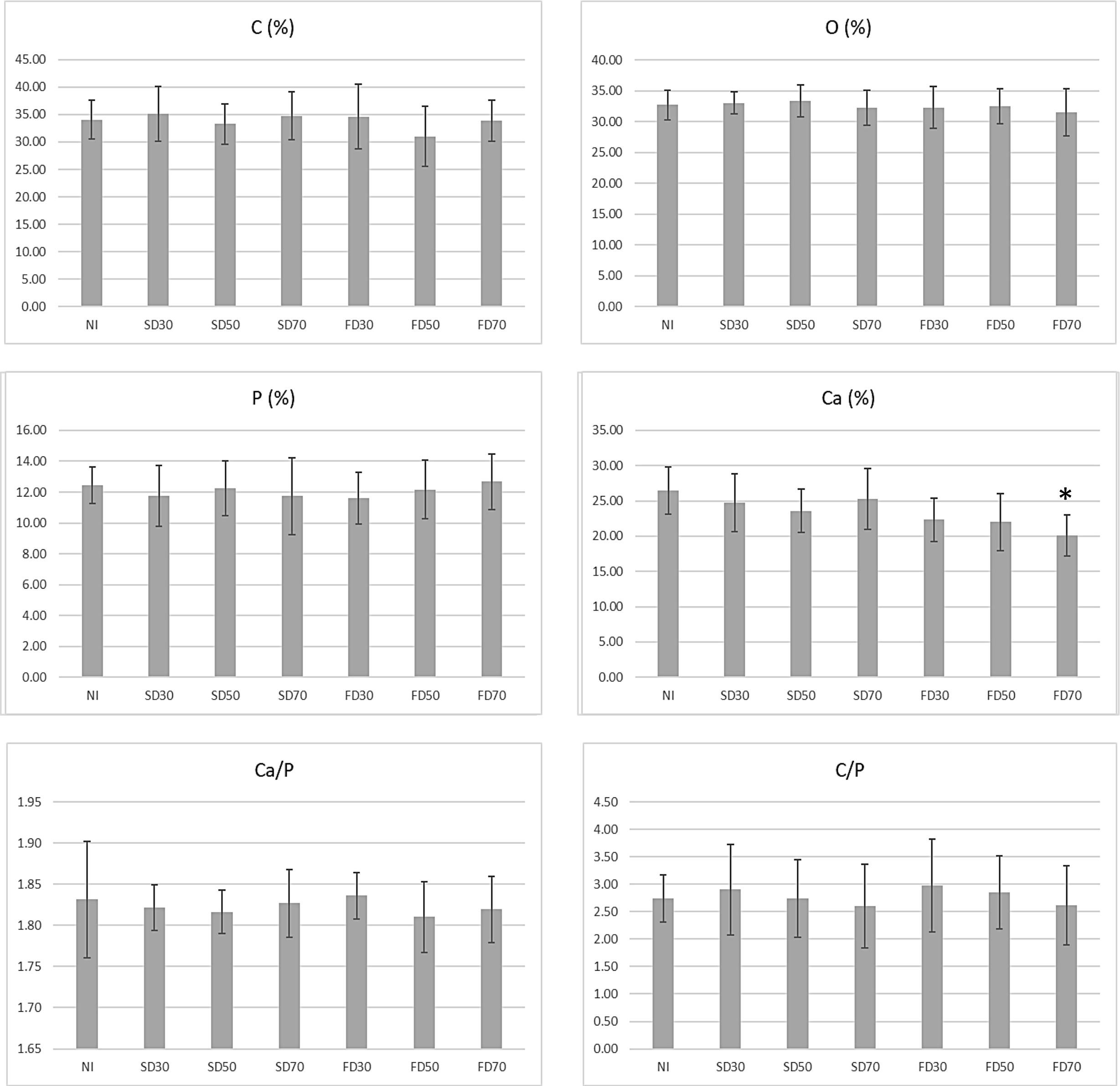


**Fig. 2** Means and standard deviations for the concentrations of each chemical element and Ca/P and C/P ratios analysed by EDS.



*Indicates a significant difference compared to the NI group at Dunnett's test ($p<0.05$)

# 4. CAPÍTULO 3

**Artigo 3:**

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Critical Review
Infection Control

# Head and neck cancer therapy-related oral manifestation management in the COVID-19 pandemic: a critical review

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**Abstract:** With the onset of the new coronavirus disease (COVID-19) pandemic, the dental treatment of patients at risk of infection has become quite challenging. In view of this, patients with head and neck cancer may present with oral complications due to anticancer therapy, making dental assistance necessary. Thus, the objective of the study was to review the literature and critically discuss important concerns about the treatment of patients with head and neck cancer during the COVID-19 pandemic. Because dental professionals are in close contact with the main viral transmission routes, this study presents recommendations for management and protection during clinical dental care. The main characteristics and transmission routes of COVID-19 are also discussed. Dental professionals should control pain and the side effects of antineoplastic treatment and use preventive measures for infection control. During this pandemic, patients with head and neck cancer should not undergo elective procedures, even if they do not have symptoms or a history of COVID-19; therefore, in asymptomatic or painless cases, only preventive actions are recommended. In symptomatic or painful cases, precautions for safe interventional treatments must be implemented by following the hygiene measures recommended by health agencies and using personal protective equipment. During health crises, new protocols emerge for cancer treatment, and professionals must act with greater attention toward biosafety and updated knowledge. It is important to offer adequate individualized treatment based on the recommendations of preventative and interventional treatments so that patients can face this difficult period with optimized quality of life.

**Keywords:** Covid-19; Coronavirus Infections; Dental Care; Head and Neck Neoplasms; Practice Management, Dental.

## Introduction

Coronavirus disease (COVID-19) is caused by a beta coronavirus, formally known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus emerged in the city of Wuhan, Hubei province, China, in December 2019 and then spread rapidly across the country and globally.[1] On March 12, 2020, the World Health Organization (WHO) declared it as a global pandemic.[2] Overall, 13,378,853 cases and

580,045 deaths due to COVID-19 were confirmed as of July 16, 2020.[2] In Brazil, which has the highest number of infected people in Latin America, 2,012,151 cases and 76,688 deaths have already been confirmed.[3]

The COVID-19 pandemic poses an unexpected public health challenge. Measures are being implemented worldwide by governments, non-governmental organizations, and individuals with an aim toward delaying the spread of the virus and, consequently, avoiding overburdening health systems.[4] The disease spreads primarily through human-to-human transmission of SARS-CoV-2 (through close contact or respiratory droplets produced when an infected person coughs or sneezes) and secondarily through contact with surfaces or objects contaminated by the virus.[5]

Hypertension and respiratory, cardiovascular, and metabolic diseases such as diabetes mellitus are important risk factors for COVID-19, particularly contributing to disease severity.[6] In addition to individuals with these comorbidities, current studies point to the elderly, obese, and those with chronic diseases as potential risk groups with repercussions on hemodynamics and immunology.[6] Patients in immunocompromised states, such as those with transplanted organs or active cancer, and using immunosuppressive agents are more susceptible and likely to progress to the most severe stage.[7,8]

Health care for patients with cancer is extremely challenging during this pandemic.[7] A recent study from China found that cancer patients with COVID-19, when compared to patients without cancer, have a higher risk of severe events, quantified by the need for admission to the intensive care unit requiring invasive ventilation or death.[9] Cancers those located in the head and neck region account for 5% of all types of cancers. The recent global cancer statistics reported an estimated 887,649 new cancer cases and 453,307 cancer-related deaths worldwide.[10] Approximately 90% of the cases are squamous cell carcinomas originating from the epithelium, with tobacco and alcohol being the most significant risk factors. Regarding the location, this type of cancer can be found on the lips, oral cavity, nasal cavity, pharynx, larynx, sinuses, and the salivary glands.[11] Head and neck cancer can be treated with a combination of modalities such as surgery, radiotherapy, chemotherapy, or immunotherapy.[8] These therapies, however, cause side effects during and after treatment, resulting in oral manifestations such as mucositis, candidiasis, xerostomia, dysphagia, dysgeusia, trismus, radiation-related caries, and osteoradionecrosis, which require intervention or close monitoring by a dental professional.[12] Thus, dentists are among the professionals with the greatest risk of contracting COVID-19 because they have close contact with patients, aerosols, saliva, and blood and use sharp contaminated instruments in clinical practice.[1,13]

Thus, the objective of the study was to review the literature and critically discuss important aspects in the treatment of patients with head and neck cancer during the COVID-19 pandemic. We also make recommendations for the management and protection of professionals during clinical dental care of such patients.

## Discussion

### SARS-CoV-2: general aspects and oral specifics

According to results from genetic and epidemiologic studies, the COVID-19 outbreak appears to have started with a single animal-to-human transmission, followed by a sustained human-to-human spread.[14] This virus has a high rate of transmissibility, and based on data from the World Health Organization, each infected person could transmit the disease to 1.4 to 2.5 people.[15] In addition, scientific evidence shows that the median incubation period is approximately 5 days and that symptoms will develop after 14 days of active monitoring or quarantine.[16]

Due to close face-to-face contact with patients and the frequent use of devices, dental professionals are repeatedly exposed to respiratory tract secretions, blood, saliva, and other contaminated body fluids, which increases the risk for viral transmission and COVID-19.[17] Transmission during dental services occurs through four main routes: direct exposure to respiratory secretions containing droplets, blood, or saliva; indirect contact with contaminated surfaces and/or instruments; inhalation of airborne viruses; and mucosal contact with infected droplets and aerosols.[17]

It is important to mention that COVID-19 greatly impacted the behavior of dental patients.[18] At the onset of the pandemic, there was a reduction in the demand for dental care. Consequently, the incidence of dental and oral infections raised from 51% to 71.9% during the COVID-19 pandemic. This change was associated with service restrictions and the overall preference for staying at home.[19] This scenario was also reflected in patients who were undergoing antineoplastic treatment.[20] Considering the need for dental monitoring to minimize the side effects of head and neck cancer, it is necessary to understand oral changes and establish dental guides to maintain oral health and quality of life for these patients during and after the pandemic.

## Oral manifestation in patients with head and neck cancer

It is a consensus that patients undergoing treatment for head and neck cancer can undergo acute and chronic changes in soft and hard tissues and sensory disturbances.[21] The treatment of head and neck cancer usually involves radiotherapy, chemotherapy, immunotherapy, and/or surgery and can affect healthy oral tissues, influencing the manifestation and progression of oral diseases and significantly affecting patients' quality of life.[22] Oral side effects of head and neck cancer treatment include oral mucositis, dysphagia, dysgeusia, and candidiasis, while the late effects include the loss of salivary gland function, trismus, radiation-related caries, and osteoradionecrosis.[12]

One of the most significant acute reactions in head and neck cancer patients is oral mucositis.[23] Radiation and/or chemotherapy causes cellular damage, which results in epithelial cell death. It is also believed that the generation of reactive oxygen species (free radicals) by radiation or chemotherapy plays a role at the beginning of the mucosal injury.[24] In its initial stage, erythema occurs due to epithelial thinning, which progresses to mucosal edema and inflammation. Over time, the mucosa becomes ulcerated and bleeds easily. The symptoms of this condition include severe pain and dysphagia.[25] The scale recommended by the WHO is mostly used to record the extent and severity of oral mucositis, which classifies mucositis in four degrees: Grade 0 (none) – No signs and symptoms; Grade I (mild) – Oral soreness, erythema; Grade II (moderate) – Oral erythema, ulcers, solid diet tolerated; Grade III (severe) – Oral ulcers, liquid diet only; Grade IV (life-threatening) – Oral alimentation impossible.[26] While the patient can still eat orally, the sensitive and inflamed oral mucosa makes it difficult to maintain oral hygiene and leads the patient to adopt a pasty diet rich in carbohydrates. These factors together increase the occurrence of periodontal disease and dental caries.[27]

Patients with dysphagia have difficulty with swallowing. Patients with head and neck cancer who have dysphagia, abnormal swallowing to severe oropharyngeal dysphagia (with the impossibility of oral feeding), can experience this problem for up to 12 months after oncological treatment.[28] Symptoms such as excessive chewing and drooling and complaint of food sticking in the throat are indicative of dysphagia. In addition, symptoms that require greater attention are those that indicate potential aspiration, such as coughing or clearing the throat before, during, or after eating.[29] During tumor removal, muscle, bone, and cartilage structures should be in a compromised state, as well as neck dissection or skull base surgery, favoring the appearance of dysphagia. The association of radiotherapy and chemotherapy may also affect the structures related to swallowing, mainly due to high doses of radiation, bilateral neck irradiation, or chemical damage to neuronal axons. The evaluation of swallowing disorders in these patients is difficult and requires the collaboration of a multidisciplinary team involving dentists, speech therapists, radiation oncologists, doctors, radiologists, and nutritionists.[30]

In turn, dysgeusia is the change or loss of taste. It can be a direct consequence of radiotherapy or chemotherapy and can also be associated with mucositis.[31] This may occur because the taste buds exposed to radiation undergo atrophy, leading to difficulty in perceiving the taste and temperature of food. The increase in the viscosity of saliva also forms a mechanical barrier, making physical contact with food difficult. Chemotherapy, however, promotes direct cytotoxicity of the papillae. Antineoplastic drugs such as cisplatin may cause taste dysfunction because they can enter the oral cavity by diffusion through the capillaries. Many patients undergoing radiotherapy together with chemotherapy report metallic or very salty taste in the absence or presence of food.[32] The restoration of taste is quite variable among patients. Perception might either gradually return to normal or be permanent in cases of severe xerostomia.[22]

Radiation-induced hyposalivation results in decreased salivary flow and is often accompanied by xerostomia, the subjective perception of oral dryness. This affects the overall homeostasis of the oral cavity due to the decrease in salivary pH, increase in salivary viscosity, and changes in salivary chemical composition.[33] Chemotherapy-induced xerostomia can start from the second day of treatment, where the drugs begin to affect cells of the salivary glands, thereby causing atrophy of the acini, necrosis, degeneration, and fibrosis. In addition to decreasing salivary flow, chemotherapy also decreases the amount of salivary amylase and IgA immunoglobulins.[34] However, use of other medications such as hypertensive drugs, antidepressants, tranquilizers, antihistamines, and diuretics might also lead to this condition.[35] These changes trigger several other complications including dry lips, dysphagia, decreased resistance to oral infections such as candidiasis and halitosis.[12] The buffering and tooth remineralization capacity is reduced, leading to the loss of the demineralization/remineralization balance and facilitating a greater propensity for dental caries.[36] In addition, xerostomia causes discomfort, pain, and irritation related to oral dryness, altering the psychological dimension of patient.[33]

One of the late side effects is radiation-related caries, a multifactorial condition with a high potential for tooth destruction. It results from radiogenic damage to the dental structure, hyposalivation, alteration of the salivary composition, and decreased pH and buffering capacity in addition to the high-carbohydrate diet that is adopted due to oral mucositis, dysphagia, and dysgeusia.[37] Its clinical pattern differs from conventional caries in that conventional caries occurs mainly in pits, fissures, and the proximal region of teeth, while radiation-related caries develops in incisal/cuspal teeth and the entire cervical region, leading to enamel delamination, destruction of the underlying dentin, and amputation of the dental crown.[38]

Trismus in head and neck cancer patients may be caused by fibrosis in the masticatory muscles after surgery or radiotherapy, and contracture in the mastication structures, including the masseter and pterygoid muscles.[12,39] The prevalence of trismus primarily depends on the location and size of the tumor, being higher in patients with tumors close to the masticatory system, such as parotid and nasopharyngeal lesions or those located in the lateral oropharyngeal cavities or the posterior oral cavity.[40] The restricted opening of the mouth negatively affects the patient because improper mastication requires changes in food consistency and poses difficulties in maintaining oral hygiene, increasing the risk for oral infections and dental problems.[41]

Osteoradionecrosis is the most serious chronic complication of radiotherapy for the treatment of head and neck cancer.[12] It usually develops in the presence of odontogenic infection or traumatic bone intervention after radiotherapy.[40] Meanwhile, medication-related osteonecrosis corresponds to bone necrosis resulting from the use of antiresorptive and antiangiogenic agents. The drugs most often associated with this condition are bisphosphonates, which are used in the treatment of several diseases, including the control of metastases and bone tumors.[42] The clinical signs and symptoms of both pathologies are quite similar and include bone necrosis, pain, dysgeusia, oroantral fistula, fetid odor, trismus, difficulty in chewing, swallowing and phonation, extraoral fistula, pathological fracture, and sepsis.[12,42] Bone exposure to the oral environment has a poor prognosis and is difficult to treat, compromising the patient's general health and quality of life.[43]

## Management of patients with head and neck cancer during the COVID-19 pandemic

Patients with head and neck cancer require special attention during the COVID-19 pandemic. As mentioned earlier, current research shows that patients older than 60 years and/or presenting with systemic conditions or diseases, such as a history of head and neck irradiation, cardiovascular disease, organ transplantation, immunosuppression, diabetes mellitus, hematological diseases, and autoimmune diseases have a worse prognosis with COVID-19.[44] A multidisciplinary treatment strategy should be designed considering antineoplastic therapy and epidemic prevention.[45] Radiotherapy and chemotherapy should be continued[46] but with schemes that reduce the number of hospital visits.[45] Thus, radiation-related side effects will continue to affect the oral health-related quality of life of cancer survivors.[12]

Oral health care professionals are now considered to be at the highest risk of infection; therefore, face-to-face appointments should be reduced.[6,47] Teledentistry (text messages, phone calls, or video calls) has provided a way of communication between the professional and the patient, which has helped decrease the risk of COVID-19.[6] This approach helps the dentist to evaluate the patient's needs, offer advice, monitor the ones undergoing treatment, and carry out follow-ups.[48] It also became a way to reduce anxiety and help patients to adapt to the new reality.[49]

During the pandemic, dental treatments are being divided into elective (non-urgent) and emergent cases.[50] The American Dental Association (ADA) recommends that elective dental procedures be avoided.[50] Thus, management is limited to the treatment of acute dental problems and the relief of symptoms of oral mucositis, xerostomia, trismus, and opportunistic infections.[51] Due to this unprecedented circumstance, the role of dentists for patients with head and neck cancer can be divided into two fronts: prevention for patients without pain and intervention for patients with pain.

## Preventative treatment: patients without pain

Even when no oral symptoms are reported, it is imperative to follow-up patients through teledentistry to prevent or minimize some oral side effects of chemoradiotherapy, regardless of the treatment stage (before, during, or after). Dental professionals should strongly recommend and encourage patients to maintain good oral hygiene. Removal of dental plaque and reduction in the population of bacteria in the oral cavity require a combination of actions. Patients should be advised to brush three times a day with a soft or ultra-soft toothbrush.[52] The use of fluoride toothpaste is essential for the remineralization of enamel and dentin.[53] Toothpastes with mint flavoring and containing sodium dodecyl sulfate (surfactant) can irritate the mucosa and should be avoided.[52] If there is an increased risk of dental caries, toothpastes with high concentrations of fluoride are available and can be prescribed.[43, 52] Flossing or interdental cleaning aids are recommended to remove dental plaque or solid debris between teeth, crowns/bridges, or dental implants. Remineralizing solutions such as sodium fluoride alcohol-free solution[20, 43] or 1% sodium fluoride gel[37] should be used daily in patients with hyposalivation to prevent and remineralize carious lesions.[43]

For plaque accumulation and reduction in *Streptococcus mutans* counts, rinsing with non-alcoholic and non-flavored chlorhexidine digluconate 0.12–0.2% solution once or twice daily is also recommended,[52,54] maintaining sustained periodontal health. Care must be taken to ensure that the chlorhexidine solution does not interfere with the fluoride solution. If there is an interaction, rising of the mouth at different times of the day should be recommended.

Salivary hypofunction and xerostomia are frequent oral adverse effects. Salivary substitutes and/or stimulants (candies, gums, and sialagogic agents such as pilocarpine) may be recommended for relief when the residual function of the salivary gland is documented.[55] It is advisable to use lip care products, humidifiers, and nasal breathing[20] and avoid harmful habits that cause mucosal irritation, such as smoking and alcohol consumption.[45] The patients should be advised about decreasing consumption of acidic and spicy food because these foods aggravate the symptoms of xerostomia and pain from mucositis.[12]

Currently, prevention of trismus and osteoradionecrosis is needed. Immediately after commencing radiotherapy, patients should perform home exercises to maintain muscular mobilization. Specific exercises, using tongue spatulas or the TheraBite device, can help maintain a maximal oral opening and jaw mobility.[56] To prevent osteoradionecrosis, oral hygiene, absence of trauma due to removable prostheses, and dental care are key.[45] Removable dental prostheses must be mechanically cleaned with or without chemical methods. At night, when not in use, the dentures should be placed in water.[52] This will prevent candidal and mucositis lesions.

Patients should be well informed about the importance of maintaining a normal and balanced diet to ensure adequate nutrition.[43] Pre-treatment evaluation of swallowing function and nutritional status is of paramount importance. Moreover, post-treatment swallowing function can benefit from pre-treatment swallowing exercises.[30] Changing the consistency of food may be inevitable due to pain and discomfort. Dietary management can be performed by a nutritionist to prevent or mitigate weight loss and reduce in overall quality of life.[57]

### Interventional treatment: patients with pain

The second front of action is intervention when patients present with oral symptoms. Precautions must be taken, and every patient should be considered a potential asymptomatic carrier of COVID-19.[58] Pre-appointment screening including medical history, must be performed before the patient visits the dental office. The entire building must be properly prepared according to ADA recommendations.[50] In the waiting room, patients should wear masks, maintain a distance of 1 meter, and have their temperature checked.[19] Adequate ventilation should be provided, and long stay in this room should be avoided. Dentists must adhere to the infection control protocol. Hand hygiene using water and soap and then using 70% hydroalcoholic solution should be followed. Additionally the standard procedure for using personal protective equipment should be followed, including their donning and doffing. Disinfection of the clinical environment before and after dental care using disinfecting products such as 62–71% ethanol, 0.5% hydrogen peroxide, and 0.1% (1 g/L) sodium hypochlorite should be carried out. Disposable physical barriers on equipment should be used. Four-handed dentistry technique should be used, and the appropriate technique for garbage disposal must be followed.[13,14,46,50]

After identifying the urgent need for dental treatment, it is important to verify the risks and benefits associated with each treatment. Management with minimally invasive procedures is essential. Before every treatment, the patient should use a mouth rinse containing 1% or 1.5% hydrogen peroxide or 0.2% povidone.[19] Dentists should use a high-volume saliva ejector and a rubber dam and decrease the use of ultrasonic instruments, high-speed handpieces, and 3-way syringes to minimize the generation of aerosols.[13,14,59] Extra-oral images such as panoramic images should be preferred over intraoral radiographs to avoid the gag or cough reflexes.[60]

Irradiated head and neck cancer patients are at risk of developing candida and herpes. During the COVID-19 pandemic, reports of any symptoms should result in a quick appointment in the dental office for proper diagnosis and prescription of medications and instructions. Infections must be treated with antifungal and antiviral drugs.[61] Dentures should be appropriately cleaned and decontaminated with the same antimicrobial agent to avoid recontamination of the oral cavity by the microbial flora of dentures.[52]

Another oral side effect of cancer treatment that can bother patients is oral mucositis. Some guidelines suggested the implementation of multiagent combination oral care protocols, benzydamine mouthwash, combined topical application and systemic administration of honey, low-level laser therapy, and photobiomodulation for the prevention of oral mucositis.[62-66] Oral cryotherapy has been indicated for the prevention of this adverse effect for patients undergoing chemotherapy and receiving bolus 5-fluorouracil.[67] As treatment for oral mucositis-associated pain, topical morphine 0.2% is indicated for head and neck cancer patients undergoing radiotherapy and chemotherapy.[68]

A multidisciplinary management approach for dysphagia presented by some patients is essential. Pain management, as well as immediate treatment of the condition, will prevent critical weight loss. In patients with a high risk of weight loss, a short period of parenteral nutrition may be indicated.[30]

Radiation-related caries has a highly destructive potential and rapid evolution and can progress to amputation of the dental crown.[69] In addition, injuries to the pulp can also occur. Thus, the strategies currently adopted to reduce the spread of microorganisms are the use of rubber dams, avoidance of rotatory instruments during cavity preparation, chemo-mechanical caries removal, and atraumatic restorative techniques.[1,54,59]

For acute periodontal disease, manual scaling is recommended, ultrasonic scalers should be avoided. If tooth extraction is necessary, atraumatic extractions should be performed, avoiding bone drilling and using resorbable sutures to minimize visits to the dental office.[20] Other measurements to reduce oral trauma should be performed to avoid osteoradionecrosis.[43] Once installed, hyperbaric oxygen has shown great improvement in its management since the 1960s;[43] however, during the pandemic, the indication for hyperbaric oxygen must be critically evaluated.

The suggestions for the management of patients with head and neck cancer undergoing antineoplastic treatment in this paper are general guidelines; therefore, the final decision will always depend on the professional's judgment, taking into account the patient's individual health situation.

## Conclusion

This critical review aimed to discuss the impact of COVID-19 on the oral health of patients with head and neck cancer undergoing antineoplastic treatment and to present suggestions to minimize the signs and symptoms of the side effects. During health crises, new protocols are emerging for cancer treatment, and professionals must act with greater attention to biosafety and updated knowledge. It is important to offer adequate individualized treatment based on the recommendations of preventative and interventional treatments. We hope to help dental professionals and patients with head and neck cancer more easily face this difficult period.

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# 5. CONCLUSÃO

Doses de 70 Gy aplicadas de forma fracionada resultaram em alterações na composição química e na microdureza tanto do esmalte quanto da dentina. O uso do fracionamento para irradiação de amostras dentais *in vitro* reproduz mais fielmente a maneira como a RT é aplicada nos pacientes oncológicos de cabeça e pescoço. Pacientes em tratamento antineoplásico podem apresentar efeitos colaterais que resultam em manifestações bucais, sendo necessário acompanhamento e/ou intervenção odontológica. Diante do cenário atual da COVID-19, tratamentos odontológicos nesse grupo de pacientes devem ser bem indicados, levando em consideração a presença ou não de sintomatologia dolorosa e a condição de saúde geral do paciente.

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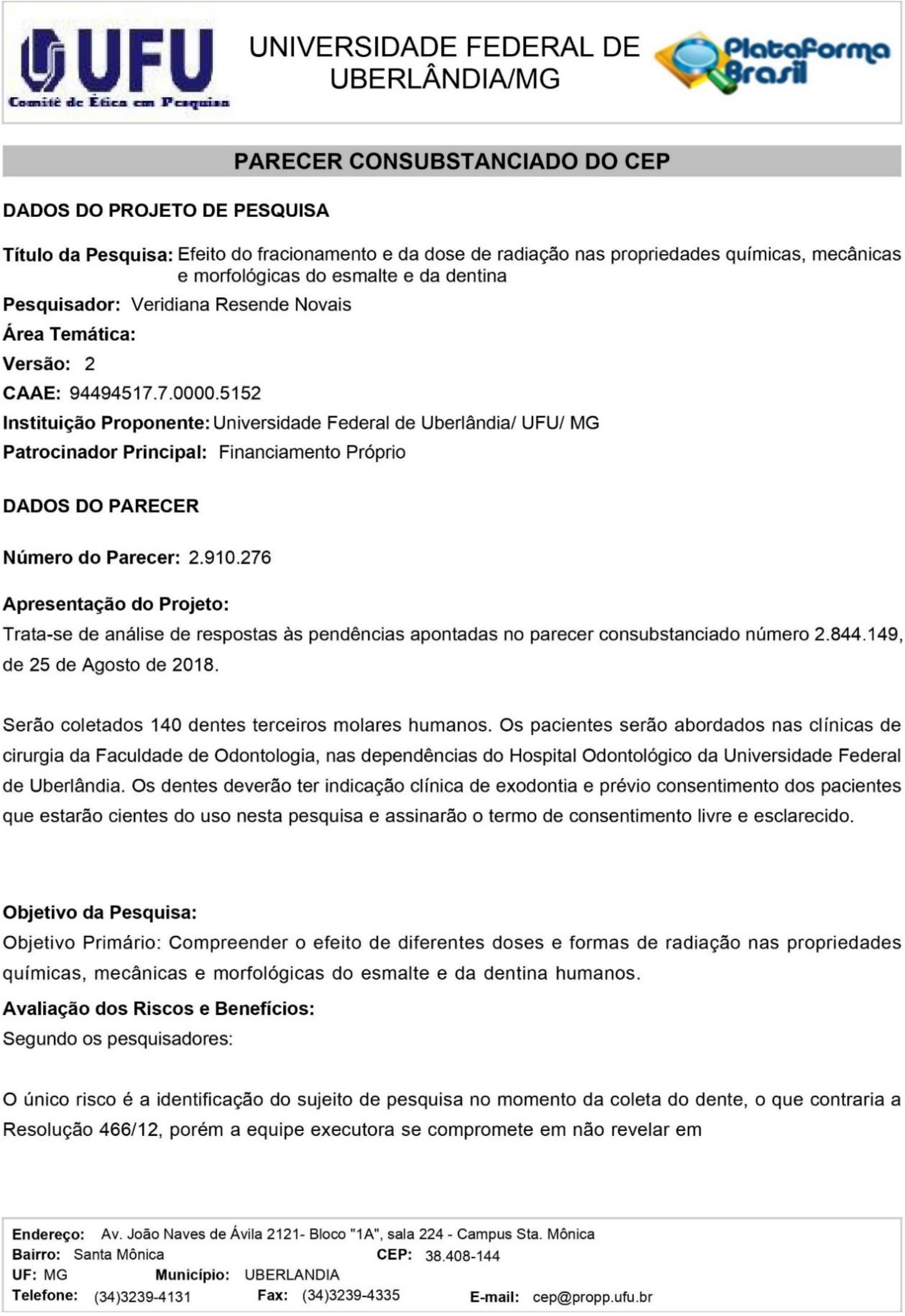
# ANEXOS

## ANEXO A – Aprovação no Comitê de Ética em Pesquisa



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Comitê de Ética em Pesquisa

UNIVERSIDADE FEDERAL DE
UBERLÂNDIA/MG

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### PARECER CONSUBSTANCIADO DO CEP

**DADOS DO PROJETO DE PESQUISA**

**Título da Pesquisa:** Efeito do fracionamento e da dose de radiação nas propriedades químicas, mecânicas e morfológicas do esmalte e da dentina

**Pesquisador:** Veridiana Resende Novais

**Área Temática:**

**Versão:** 2

**CAAE:** 94494517.7.0000.5152

**Instituição Proponente:** Universidade Federal de Uberlândia/ UFU/ MG

**Patrocinador Principal:** Financiamento Próprio

**DADOS DO PARECER**

**Número do Parecer:** 2.910.276

**Apresentação do Projeto:**

Trata-se de análise de respostas às pendências apontadas no parecer consubstanciado número 2.844.149, de 25 de Agosto de 2018.

Serão coletados 140 dentes terceiros molares humanos. Os pacientes serão abordados nas clínicas de cirurgia da Faculdade de Odontologia, nas dependências do Hospital Odontológico da Universidade Federal de Uberlândia. Os dentes deverão ter indicação clínica de exodontia e prévio consentimento dos pacientes que estarão cientes do uso nesta pesquisa e assinarão o termo de consentimento livre e esclarecido.

**Objetivo da Pesquisa:**

Objetivo Primário: Compreender o efeito de diferentes doses e formas de radiação nas propriedades químicas, mecânicas e morfológicas do esmalte e da dentina humanos.

**Avaliação dos Riscos e Benefícios:**

Segundo os pesquisadores:

O único risco é a identificação do sujeito de pesquisa no momento da coleta do dente, o que contraria a Resolução 466/12, porém a equipe executora se compromete em não revelar em

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**Telefone:** (34)3239-4131 **Fax:** (34)3239-4335 **E-mail:** cep@propp.ufu.br

nenhum momento da pesquisa a identidade dos pacientes que aceitarem participar.

Os resultados obtidos possibilitarão aos cirurgiões dentistas compreenderem de maneira mais aprofundada as alterações que ocorrem nos tecidos dentais desses pacientes, o que guiaria os profissionais quanto à melhor forma de tratar e restabelecer a saúde bucal dos mesmos.

**Comentários e Considerações sobre a Pesquisa:**

Pesquisa acadêmica visando avaliar o efeito de doses e formas de irradiação no esmalte e na dentina humanos.

**Considerações sobre os Termos de apresentação obrigatória:**

Adequados.

**Recomendações:**

Não há.

**Conclusões ou Pendências e Lista de Inadequações:**

As pendências apontadas no parecer consubstanciado número 2.844.149, de 25 de Agosto de 2018, foram atendidas.

De acordo com as atribuições definidas na Resolução CNS 466/12, o CEP manifesta-se pela aprovação do protocolo de pesquisa proposto.

O protocolo não apresenta problemas de ética nas condutas de pesquisa com seres humanos, nos limites da redação e da metodologia apresentadas.

**Considerações Finais a critério do CEP:**

Data para entrega de Relatório Parcial ao CEP/UFU: Março de 2020.

Data para entrega de Relatório Final ao CEP/UFU: Março de 2021.

OBS.: O CEP/UFU LEMBRA QUE QUALQUER MUDANÇA NO PROTOCOLO DEVE SER INFORMADA IMEDIATAMENTE AO CEP PARA FINS DE ANÁLISE E APROVAÇÃO DA MESMA.

O CEP/UFU lembra que:

a- segundo a Resolução 466/12, o pesquisador deverá arquivar por 5 anos o relatório da pesquisa e os Termos de Consentimento Livre e Esclarecido, assinados pelo sujeito de pesquisa.

b- poderá, por escolha aleatória, visitar o pesquisador para conferência do relatório e

documentação pertinente ao projeto.
c- a aprovação do protocolo de pesquisa pelo CEP/UFU dá-se em decorrência do atendimento a Resolução CNS 466/12, não implicando na qualidade científica do mesmo.

Orientações ao pesquisador :
• O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 466/12 ) e deve receber uma via original do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado.
• O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS 466/12), aguardando seu parecer, exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade de regime oferecido a um dos grupos da pesquisa que requeiram ação imediata.
• O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS 466/12). É papel de o pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.
• Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projetos do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprobatório do CEP, para serem juntadas ao protocolo inicial (Res.251/97, item III.2.e).

**Este parecer foi elaborado baseado nos documentos abaixo relacionados:**

| Tipo Documento | Arquivo | Postagem | Autor | Situação |
|---|---|---|---|---|
| Informações Básicas do Projeto | PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1010488.pdf | 29/08/2018 11:23:22 | | Aceito |
| Parecer Anterior | Resposta_ao_comite_de_etica.pdf | 29/08/2018 11:19:17 | Veridiana Resende Novais | Aceito |
| Declaração de Instituição e Infraestrutura | Declaracao_Instituicao_Coparticipante3.pdf | 26/07/2018 10:45:22 | Veridiana Resende Novais | Aceito |

| | | | | |
|---|---|---|---|---|
| Projeto Detalhado / Brochura Investigador | Projeto_de_Pesquisa_CEP.pdf | 26/07/2018 10:44:44 | Veridiana Resende Novais | Aceito |
| Folha de Rosto | Folha_de_rosto.pdf | 12/07/2018 19:56:41 | Veridiana Resende Novais | Aceito |
| Declaração de Instituição e Infraestrutura | Solicitacao_do_pesquisador_para_a_instituicao.pdf | 11/07/2018 12:37:24 | Veridiana Resende Novais | Aceito |
| Outros | Links_para_Curriculos_Lattes_dos_pesquisadores.pdf | 11/07/2018 12:34:28 | Veridiana Resende Novais | Aceito |
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| TCLE / Termos de Assentimento / Justificativa de Ausência | TCLE.pdf | 11/07/2018 12:25:24 | Veridiana Resende Novais | Aceito |

**Situação do Parecer:**
Aprovado

**Necessita Apreciação da CONEP:**
Não

UBERLANDIA, 23 de Setembro de 2018

**Assinado por:**
**Karine Rezende de Oliveira**
**(Coordenador(a))**

## ANEXO B – Normas do periódico Archives of Oral Biology

# ARCHIVES OF ORAL BIOLOGY

A Multidisciplinary Journal of Oral & Craniofacial Sciences

**AUTHOR INFORMATION PACK**

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**ISSN:** 0003-9969

## DESCRIPTION

*Archives of Oral Biology* is an international journal which aims to publish papers of the highest scientific quality in the oral and craniofacial sciences including:
Developmental biology Cell and molecular biology Molecular genetics Immunology Pathogenesis Microbiology Biology of dental caries and periodontal disease Forensic dentistry Neuroscience Salivary biology Mastication and swallowing Comparative anatomy Paeleodontology *Archives of Oral Biology* will also publish expert reviews and articles concerned with advancement in relevant methodologies. The journal will only consider clinical papers where they make a significant contribution to the understanding of a disease process.Journal Metrics

## AUDIENCE

Oral biologists, physiologists, anatomists, pathologists.

## IMPACT FACTOR

2019: 1.931 © Clarivate Analytics Journal Citation Reports 2020

## ABSTRACTING AND INDEXING

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CABI Information
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Gale Database of Publications & Broadcast Media
Global Health
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Pascal Francis
RTECS (Registry of Toxic Effects of Chemical Substances)
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SPORTDiscus
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UnCover
Zoological Record
BIOSIS Toxicology
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Archives of Oral Biology is an international journal which aims to publish papers of the highest scientific quality reporting new knowledge from the orofacial region including:
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- molecular genetics
- immunology
- pathogenesis
- microbiology
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- forensic dentistry
- neuroscience
- salivary biology
- mastication and swallowing
- comparative anatomy
- paeleodontology

Archives of Oral Biology will also publish expert reviews and articles concerned with advancement in relevant methodologies. The journal will consider clinical papers only where they make a significant contribution to the understanding of a disease process.

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## Article structure

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