UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

TAMIRES DE LUCCAS BUENO

Effect *in vitro* and *in situ* of proanthocyanidin-based dentifrices at the demineralized organic matrix submitted to erosion and dental abrasion

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Efeito *in vitro* e *in situ* de dentifrício à base de proantocianidina na matriz orgânica desmineralizada submetida à erosão e abrasão dentária

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Orientador: Prof. Dr. Heitor Marques Honório

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RESUMO

Efeito *in vitro* e *in situ* de dentifrício à base de proantocianidina na matriz orgânica desmineralizada submetida à erosão e abrasão dentária

Este estudo teve como objetivo avaliar "*in vitro* e *in situ*" o efeito de dentifrício experimental à base de Proantocianidina na matriz orgânica desmineralizada, após ciclos de erosão e abrasão dentária. O estudo in vitro foi realizado com 50 espécimes obtidos de dentes bovinos, limpos e cortados em blocos de dentina 4x4x2 mm, polidos com tiras de lixa e com feltro. Os espécimes forma divididos em 5 grupos (N=10), de forma randomizada: G1- placebo (controle negativo); G2 – clorexidina a 0, 012% (1° controle positivo); G3 - NaF 1110 ppm (2° Controle Positivo); G4- proantocianidina 10% (1° grupo teste); G5- proantocianidina + flúor (2° grupo teste). Foram realizadas 3 ciclagens erosiva, 3x/dia por 5 min cada uma, por 5 dias. Logo após a segunda e terceira ciclagem, a escovação dos espécimes foram realizadas em uma máquina de escovação com uma carga 150g por 30s. O estudo in situ foi duplo-cego cruzado, realizado em 5 fases de 5 dias cada, com 10 voluntários saudáveis, que usaram 5 dispositivos palatais (1 para cada fase) com 4 amostras de dentina. Assim como realizado no in vitro, os blocos de dentina foram divididos de forma randomizada nos 5 grupos. Os participantes submeteram os dispositivos palatais em ciclagem erosiva, conforme o descrito para o estudo *in vitro*. No entanto, a escovação foi realizada pelos próprios participantes com escova elétrica por 30s. Tanto *in vitro*, quanto *in situ* a análise de microdureza foi realizada para seleção dos espécimes, já a perfilometria foi conduzida nos tempos iniciais e finais, sendo efetuadas cinco medidas para cada período. No estudo *in vitro* os dados foram analisados pelo teste de Kruskal-Wallis, Análise de Variância a um critério (ANOVA), seguido do teste de Tukey ($p < 0,05$). Sendo os resultados obtidos: G1- 1,68 (1,52-2,08); G2- 1,25 (1,05-1,44); G3- 0,87 (0,74-1,03); G4- 0,48 (0,40-0,60); G5- 0,58 (0,55-0,62). O grupo G4 e G5 não houve diferença significativa, apresentando menor perda de dentina quando comparados aos demais grupos e G1 apresentou o maior degaste. Para o estudo *in situ*, os dados foram analisados pela ANOVA um critério, seguida do teste de LSD Fisher ($p < 0,05$): G1- 1,76 (± 0,55); G2 - 1,19 (± 0,42); G3-1,29 (± 0,34); G4- 0,93 (± 0,38); G5 - 0,82 (± 0,34). Não apresentou diferença significativa entre os grupos G4 e G5, mas apresentou menor perda de dentina quando comparado a todos os outros grupos. Os dentifrícios de PA e sua combinação com dentifrícios fluoretados podem ser uma alternativa promissora para pacientes que sofrem com a erosão dentinária, evitando a perda mineral.

Palavras-Chaves: Dentina. Erosão. Abrasão. Metaloproteinases da Matrix. Dentifrícios.

ABSTRACT

Effect *in vitro* and *in situ* of proanthocyanidin-based dentifrices at the demineralized organic matrix submitted to erosion and dental abrasion

The objective of this study was to evaluate, in vitro and in situ, the effect of experimental dentifrice based on Proanthocyanidin in the organic demineralized matrix after erosive and abrasive challenges. The in vitro portion of the study was performed using 50 dentin specimens obtained from bovine teeth (4x4x2 mm), randomly divided in five groups after microhardness test (ranging from:41) (N=10 per group): G1- placebo (negative control); G2- 0.012% chlorhexidine (1st positive control); G3- NaF 1110 ppm fluoride (2nd positive control); G4- 10% purified proanthocyanidin (1st test); G5- fluoride + proanthocyanidin (2nd test). Erosive challenge was performed using an acid beverage (Coca-cola®), 3x per day during 5min each, for 5 days. The abrasion challenge was performed after the first and third erosive cycles using a mechanical brushing simulator with 150g of load for 30s in each specimen covered with the respective dentifrice slurry. In between the erosive/abrasive challenges, specimens were stored artificial saliva. The in situ portion of the study followed a crossover, double-blinded, design, and was performed in 5 phases of 5 days each, with 10 healthy volunteers who wore 5 palatal devices (1 for each phase) with 4 dentin specimens each. As for the in vitro portion, dentin blocks were randomly divided into 5 groups and submitted to the same erosive challenges. For abrasive challenge, brushing was performed by the volunteers using an electric brush for 30s. For both in vitro and in situ portions, the profilometry was conducted at the initial and final time points, with five measurements for each period. In vitro data were analyzed using Kruskal-Wallis test, one-way ANOVA, following by Tukey test ($p < 0.05$), and showed G4 and G5 with the lowest wear with median and Q1-Q3 of 0.48 (0.40-0.60) and 0.58 (0.55-0.62), respectively, followed by G3- 0.87 (0.74-1.03), G2- 1.25 (1.05-1.44), and G1- 1.68 (1.52-2.08). In situ data were analyzed by one-way ANOVA and Fisher's LSD test ($p < 0.05$), and showed similar trend of results: G4 and G5 showing the lowest wear 0.93 (± 0.38) and 0.82 (± 0.34) respectively, followed by G2 – 1.19 (± 0.42) and G3-1.29 (± 0.34), and G1- 1.76 (± 0.55) showing the highest wear. PA dentifrices and its combination with fluoride dentifrice can be an alternative to prevent mineral losses during erosive and abrasive challenges.

Keywords: Dentin. Erosion. Abrasion. Matrix metalloproteinases. Dentifrices.

LIST OF ILLUSTRATIONS

PAPER 1

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LIST OF ABBREVIATIONS AND ACRONYMS

g	Grams
h	Hours
min	Minutes
ml	Milliliters
mm	Milimeters
µm	Micrometers
CHX	Chlorhexidine
NaF	Sodium fluoride
1_{st}	First
2_{nd}	Second
G1	Placebo dentifrice group
G2	0.012% chlorhexidine dentifrice group
G3	NaF 1110 ppm fluoride dentifrice group
G4	10% purified proanthocyanidin dentifrice group
G5	Proanthocyanidin dentifrice + Fluoride group
s	seconds
DOM	Demineralized organic matrix
pH	Hydrogenic potential
MMP	Metalloproteinases
°C	Celsius degree
Al_2O_3	Aluminium oxide
#	Number
Ca^{2+}	Calcium
Zn^{2+}	Zinc
PA	Proanthocyanidin
CAPES	Brazilian Federal Agency for Support and Evaluation of Graduate Education
CNPq	National Council for Scientific and Technological Development

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1 Introduction

1 INTRODUCTION

In the last years, the prevalence of non-carious lesions has been increasing as a result of the increase in life expectance and maintenance of teeth in the oral cavity. Such lesions are characterized for mineral losses not caused by bacteria or bacterial subproducts (SHELLIS; FEATHERSTONE; LUSSI, 2014) and can present different classifications according with its etiology and clinical characteristics: abfraction, attrition, abrasion, and erosion.

The abfraction consists in the mineral loss characterized by occlusal overloads and stress concentration at cervical areas (ECCLES, 1982; GRIPPO, 1991). Attrition is a pathology caused by the contact between teeth (EISENBURGER; ADDY, 2002). Tooth abrasion occurs through mechanical forces during tooth brushing (which is related to brush bristle type, forces applied by patient, and dentifrices' abrasiveness) (ECCLES, 1982; RIOS *et al.*, 2006; RIOS *et al.*, 2007; MAGALHAES *et al.*, 2009; WEST; JOINER, 2014). Dental erosion consist in loss of mineral content as a result of intrinsic or extrinsic acids. Extrinsic acids are related with dietary habits such as frequent consumption of acidic beverages; intrinsic acids are produced by the patient's body, and tooth mineral losses can happen as a result of regurgitation and reflux disorders (i.e. anorexia and bulimia) (IMFELD, 1996; MEURMAN; TEN CATE, 1996; CARVALHO *et al.*, 2014; SHELLIS; FEATHERSTONE; LUSSI, 2014).

Tooth wear will start in enamel and, if the causes are not removed/controlled, the lesions will tend to progress into dentin, where its progression might be faster as result of the different tooth structures' composition (SHELLIS *et al.*, 2014). The dental enamel has a higher mineral composition (vol.85%), represented by crystals of hydroxyapatite arranged in prisms and it can suffer dissolution by acid substances, that can presented different concentrations of pH, making them fragile, soft and susceptible to wear, depending on the amount of acid challenge (HONÓRIO *et al.*, 2008; RIOS *et al.*, 2008; HONÓRIO *et al.*, 2010; SHELLIS *et al.*, 2011; GANSS; LUSSI, 2014). Dentin is a more complex structure, composed by inorganic components/apatite (47%), organic components (33%), and water (20%); and start to suffer dissolution at higher pH (5.5). The organic part consists of type I collagen (90%) and dentin phosphoproteins, proteoglycans and glycosaminoglycans (10% - non-collagen component) (SILVERSTONE; HICKS, 1985; PASHLEY *et al.*, 2004; MAGALHÃES *et al.*, 2009).

Mineral loss starts in enamel and tend to progress into dentin, when such lesions usually will start to be accompanied by dentin hypersensitivity (WEST *et al.*, 2013; Wara-

Aswapati *et al.*, 2005). Dental erosion occurs centripetally in dentin, expanding in the peritubular area and continuing towards the intertubular area (GANSS *et al.*, 2009; BUZALAF *et al.*, 2012). In addition to the acid-induced mineral losses, the collagenous organic matrix is also degraded by enzymes (i.e. metalloproteinases/MMP 2, MMP 8, MMP 9, and cathepsins K) (BUZALAF *et al.*, 2014; TJADERHANE *et al.*, 2015; TERSARIOL *et al.*, 2010).

The MMPs are activated in lower pH (during ingestion or contact with acids substances) however, these enzymes only degrade the collagen present at demineralized organic matrix (DOM) after the salivary neutralization (TJADERHANE *et al.*, 2015; ZARELLA *et al.*, 2015). Consider an important barrier in diffusion of acids, the DOM minimize the mineral loss preventing the demineralization of subjacent dentin (BUZALAF *et al.*, 2012; BUZALAF *et al.*, 2015).

Clinically, it is almost impossible to determine a single etiologic factor for non-carious lesions, being association between erosion and abrasion the most frequent causes, modulated by frequency, duration, and type/intensity of force. Brushing consists in the mainly abrasive challenge and it is also influenced by abrasives present in dentifrices, frequency, duration, and applied force, as well as toothbrush's characteristics (i.e. hardness) (DYER *et al.*, 2000; MAGALHÃES *et al.*, 2014).

It is noteworthy that roots/cement can also be susceptible to those lesions as a result of periodontal disease and/or gingival trauma, leaving the cervical area exposed to the chemical and/or physical challenges (MAGALHÃE *et al.*, 2012). Scientifics evidences proved that extrinsic factors, as well as higher frequency of acids food by patients are associate with erosive tooth wear (ECCLES; JENKINS, 1974; NUNN, 1996; ZERO, 1996; CARVALHO; MESTRINHO, 2014).

In view of the above, it is important to establish an intervention capable of avoiding collagen degradation and stabilize erosive tooth wear (Brackett *et al.*, 2015), as well as minimize dentin sensitivity, contributing for prevention of necessity of more costly and time-consuming treatments (SERRA *et al.*, 2009).

Studies have been focusing in prevent enzymes-driven degradation of dentin collagen matrix using the inhibitory effects of fluorides and chlorhexidine, as an approach to decrease lesion's progression (BUZALAF *et al.*, 2014). Nonetheless, fluoride is not capable of

complete inhibit MMPs and its association with other inhibitors may be more efficient (KATO *et al.*, 2014).

Other MMPs inhibitors often includes natural products derived from fruits, vegetables, seeds, and nuts, such as the proanthocyanidins (PA) which presents with flavan 3 – ol subunit linked with C4-C6 (C8) (HAN *et al.*, 2003; KENNEDY; TAYLOR, 2003). Scientific evidences show that PA decrease collagen degradation and water sorption, as well as increase mechanical proprieties of dentin matrix (CASTELLAN *et al.*, 2010; CASTELLAN *et al.*, 2011). In addition, PA presents affinity with proteins rich in proline (as collagen), be able in synthetize of the collagen (HAN *et al.*, 2003), and also decrease endogeans proteases, inactivating and protecting areas of cleavage inside of collagen (Bedran-Russo *et al.*, 2014). The main mechanism of action consists in stabilization of dentin matrix through remineralization (forming calcium- PA complexes), resisting bacterial or enzymatic-driven degradation (BEDRAN-RUSSO *et al.*, 2011; LIU *et al.*, 2013; LIU; WANG, 2013; BEDRAN-RUSSO *et al.*, 2014; AYDIN *et al.*, 2019).

Based on the necessity of preventive treatments and on the promising properties of PA, the present thesis had as objectives to evaluate the effect of incorporating PA on dentifrices (associated or not with fluorides) and its action on dentin submitted to erosive and abrasive challenges. The primary purpose of paper 1 was to evaluate, in vitro, the behavior of different dentifrices in DOM after erosive and abrasive challenges. Paper 2 shared the same goal, but using an *in situ* evaluation.

3 Discussion

3 DISCUSSION

Dental erosion has been extensively studied due to its increased prevalence in the population and associated clinical complications (i.e. dentin hypersensitivity). However, clinical studies present limitations related to quantification of the mineral loss (WEST *et al.*, 2011). Therefore, *in vitro* and *in situ* studies have been performed to overcome those limitations in a intraoral-like environment (WEST *et al.*, 2011).

The in vitro portion had as advantages to be cheaper, quicker, and more controlled than other studies designs, serving as a pilot study previous *in situ* study. It also served as reference for sample size calculation and diagnosis of possible outcomes (WEST *et al.*, 2011; WEST *et al.*, 2017). The *in situ* portion was designed to prove the results obtained during the in vitro portion, but using an intraoral environment after the erosive and/or abrasive challenges (KLONT; TEN CATE, 1991; WEST *et al.*, 2011). The adopted protocol consisting of not using the appliances overnight was selected in order to improve results' reliability and volunteers' comfort (WEST *et al.*, 2011; ALENCAR *et al.*, 2016; MENDONCA *et al.*, 2017). Both analysis (*in vitro* and *in situ*) showed that PA-based dentifrices, associated or not with fluorides reduced dentin wear. The null hypothesis was rejected using both study's designs.

Dentifrices containing inhibitory agents could consist in an easily adopted, widespread, and cost-effective treatment modality to increase the resistance to erosive and abrasive challenges (GANSS *et al.*, 2014; MAGALHAES *et al.*, 2014).

Different acids and protocols are used to promote dental wear and simulate erosive and abrasive challenges (LUSSI *et al.*, 1995; REDDY *et al.*, 2016). The Cola Cola soft drink presents a lower pH of 2.5 and is widely consumed acid drink (RIOS *et al.*, 2006), and was used in both studies. Between erosive and abrasive challenges, the samples were stored in artificial saliva (*in vitro*), and in natural saliva (*in situ*), during a minimum of 2h before the following erosive and/or abrasive cycles. Although the results had been similar between both studies, the natural saliva can be influenced the small difference at values of wear as the salivary MMPs are active in lower pH and contribute with dentin MMPs for degradation of DOM (BUZALAF *et al.*, 2015). Abrasives cycles were performed twice during 30s per cycle. Although the in vitro study allowed standardization of load at 150g, the *in situ* study relied on the pressure applied by the volunteers.

Root bovine dentin were used in both studies as it is considered similar to human dentin when considering MMPs-2 and 9 activity, as well as due for being easily available (KATO *et al.*, 2011).

The mainly response variable was profilometry, which allows graphic representation of the sample surface (GANSS *et al.*, 2007), however, the measure in dentin must be under wet conditions for maintenance the dentin collagen of matrix (ATTIN *et al.*, 2009).

The G1/placebo dentifrice showed the highest dentin wear for both studies confirming that the absence of active principles capable of inhibit the MMPs in fact does not prevent wear (HANNAS *et al.*, 2016). In contrast, CHX is considered gold standard for dental erosion in dentine inhibiting the degradation of collagen by MMPs (TJADERHANE *et al.*, 2015). CHX chelates with calcium with metal ions, competing with the MMPs (GENDRON *et al.*, 1999; BRESCHI *et al.*, 2018), and avoiding the degradation of fibrillar collagen by MMPs enzymes and cysteine cathepsins (SCAFFA *et al.*, 2012).

Sodium fluoride present in dentifrices is well known for prevention of dental caries, but it also presents action to prevent dental erosion through remineralization and inhibition of MMPs (especially MMPs- 2 and 9) through competition with Ca2+ ion (KATO *et al.*, 2014; BUZALAF *et al.*, 2014; MAGALHAES *et al.*, 2014; BRESCHI *et al.*, 2018). Although the higher the concentration of NaF, the lower the degradation of the demineralized dentin (BRACKETT *et al.*, 2015), the resent study used 1100ppm as it is commonly commercialized. For both studies, CHX and Fluoride-containing dentifrices reduced dentin wear when compared with the control group.

Nevertheless, PA-containing dentifrices showed the lowest dentin wear among all groups for both studies, probably due to its capabilities of stabilizing the DOM and avoid the collagen degradation (BOTEON *et al.*, 2017) through crosslinking and biomodifications of dentin structure (BEDRAN-RUSSO *et al.*, 2011; CASTELLAN *et al.*, 2011; AYDIN *et al.*, 2019). Such biomodifications improve the mechanical proprieties, promotes dentin mineralization, and stimulate collagen synthesis (CASTELLAN *et al.*, 2010; BEDRAN-RUSSO *et al.*, 2014; BEDRAN-RUSSO *et al.*, 2014; EPASINGHE *et al.*, 2017; BALALAIE *et al.*, 2018; AYDIN *et al.*, 2019). In addition, it can inactivate cathepsins cysteines and approximately 89% of the MMPs (EPASINGHE *et al.*, 2013; SCHEFFEL *et al.*, 2014).

PA can be extracted from several natural sources and, for the present study, it was derived from grape seeds as proposed previously (BEDRAN-RUSSO et al., 2011). The association of MMP inhibitors with fluoridated dentifrice as an attempt to reduce dental erosion (BUZALAF et al., 2012) has been confirmed with these studies. The PA dentifrice was effective for reduction of dentin wear being an excellent solution for patients suffering erosion and/or abrasion used daily.

4 Conclusion

4 CONCLUSION

It can be concluded that PA dentifrices, associated or not with fluorides, promoted the reduction of dentin mineral loss after erosion and abrasion challenges both in situ and in vitro.

Incorporation of PA in dentifrices may be interesting as a widespread, cost-effective preventive treatment against dental wear.

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Appendixes

APPENDIX A- DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS-PAPER-1

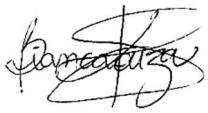
DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article EFFECT OF PROANTHOCYANIDIN-BASED DENTIFRICES ON DENTIN-WEAR – IN VITRO STUDY will be included in Thesis of the student Tamires de Luccas Bueno was not used and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, 04 de junho de 2019.

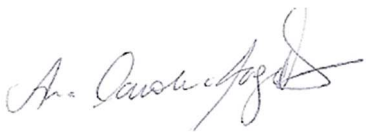
Tamires de Luccas Bueno
Author

Bianca Katsumata de Souza
Author



Fabio Antonio Rizzante
Author

Ana Carolina Magalhães
Author



Daniela Rios
Author

Heitor Marques Honório
Author

APPENDIX B- DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS-PAPER-2

DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article EVALUATION OF PROANTHOCYANIDIN-BASED DENTIFRICES ON DENTIN-WEAR AFTER EROSION AND DENTAL ABRASION - IN SITU STUDY will be included in Thesis of the student Tamires de Luccas Bueno was not used and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, 04 de junho de 2019.

Tamires de Luccas Bueno
Author

Tamires Alves Pereira da Silva
Author

Fabio Antonio Rizzante
Author

Ana Carolina Magalhães
Author

Daniela Rios
Author

Heitor Marques Honório
Author

APPENDIX C- TERM OF CONSENTING

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós Prof. Dr Heitor Marques Honório e Tamires de Luccas Bueno, responsáveis pela pesquisa intitulada **"Efeito in vitro e in situ de dentifrícios na matriz orgânica desmineralizada submetida a erosão e abrasão dentária",** convidamos você aluno de Pós- Graduação (mestrado e doutorado) na faixa etária de 21- 35 anos, a participar desse nosso estudo.

Esta pesquisa tem como objetivo desenvolver uma "pasta de dente" a base de compostos naturais para diminuir desgaste da estrutura do dente para pacientes que fazem uso de alimentos ácidos, que tenham problemas gástricos ou que são expostos a produtos químicos em seu ambiente de trabalho.

A pesquisa terá 5 (cinco) etapas de 5 (cinco) dias cada, tendo um intervalo de descanso de 7 (sete) dias entre elas.

Aceitando participar dessa pesquisa você será moldado para a realização de uma placa que irá no "céu da boca". Esta plaquinha terá 4 (quatro) pedaços de dente de boi, devidamente esterilizados e que você fará durante cinco dias sem interrupção. O aparelho será instalado um dia antes do início da pesquisa, à noite, após a última higiene, para permitir formação de película adquirida. Você terá que usá-lo durante o dia e noite, no entanto, você poderá tirá-lo por meia hora no período da manhã, uma hora no almoço, meia hora no meio da tarde e uma hora no período da janta para se alimentar.

Durante o período experimental você deverá colocar o aparelho por inteiro, quatro vezes ao dia, durante os períodos disponíveis para alimentação, por 5 minutos, em um copo contendo 200ml da bebida do estudo (Coca-Cola). Sendo o líquido trocado a cada imersão e mantido em temperatura ambiente.

Você deverá fazer escovação dos blocos com uma escova elétrica por 30 segundo em cada bloco, após a imersão no líquido, realizando nos períodos destinados ao almoço e a janta, com a pasta testada que estará presente em seu Kit.

Terminando cada etapa os aparelhos serão devolvidos ao responsável da pesquisa, e você ficará sete dias sem o uso do aparelho para que a próxima etapa seja iniciada, sendo os mesmos procedimentos realizados em cada etapa, conforme descrito à cima, no entanto somente a pasta será diferente.

Para realizar as etapas você receberá um kit contendo: uma escova, pasta de dente e fio dental para higiene bucal quando estiver com o aparelho fora da boca (que serão de seu domínio ao final da pesquisa). Para escovação dos blocos presentes na placa, você receberá uma escova elétrica à bateria, pasta de dente a ser testada (que serão devolvidos no final de cada etapa). O kit contará com um copo para imersão em solução erosiva (Coca-cola), gaze para envolver o aparelho, que será umedecida com água de abastecimento (Bauru- 0.7 ppm F) e um estojo para armazenar o aparelho, quando este estiver fora da boca.

Você utilizará o aparelho durante 25 dias no total, não havendo nenhum risco de dano aos seus dentes, já que o desafio erosivo será realizado com o dispositivo fora da sua boca. Durante o período de uso do aparelho, você não deverá consumir bebidas ou alimentos ácidos (exceto água). O uso do mesmo não causará dor à você, mas poderá ocorrer algum desconforto ou dificuldade na pronúncia de algumas palavras nas primeiras horas, que se restabelecerá normalmente após a adaptação à condição de existência de aparelhos dentro da boca. A utilização do aparelho não afetará sua qualidade de vida ou rotina, pois será semelhante ao uso de aparelhos que consertam dentes tortos.

Você **não** terá benefício direto, já que todo o tratamento será realizado na placa utilizada por você. Porém, **tendo resultado satisfatório** com as pastas testadas, e em caso de necessidade de uso, este **será disponibilizado e prescrito** a você. Os resultados obtidos com essa pesquisa poderão colaborar com desenvolvimentos de protocolos que ajudarão em futuras pesquisas sobre dentes com desgaste (erosão). Embora **não seja previsto nenhum risco**, incômodos no momento da moldagem e do uso do dispositivo podem ocorrer, no entanto, em caso de eventuais danos decorrentes da pesquisa, **os pesquisadores garantem indenização aos convidados**. Verificando-se a necessidade de algum tratamento não relacionado com a pesquisa, você receberá tratamento nas dependências dessa instituição.

O uso do dispositivo é de suma importância, pois permite realizar o estudo imitando os eventos que ocorrem na boca de forma fiel, sem trazer nenhum efeito colateral aos dentes dos convidados por que os aparelhos são utilizados.

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A sua **participação será voluntária**, isto é, a qualquer momento você poderá recusar –se a participar da pesquisa interrompendo o uso do aparelho e retirar seu consentimento. Sua recusa **não** terá nenhum prejuízo em sua relação com o pesquisador, nem com a instituição. Asseguro que **sua participação nessa pesquisa será sigilosa durante toda a pesquisa**, assim como todos os seus dados pessoais não serão divulgados em eventos, congressos ou em revista. Você **não terá nenhum custo** por participar dessa pesquisa, pois todo o material será devidamente fornecido por mim, e esta será realizada enquanto o convidado estiver presente na Faculdade, em horários que não interfiram em suas atividades.

Estando de acordo em participar da pesquisa o aceite será formalizado através da assinatura do presente "TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO", em dua vias, sendo que uma delas será entregue a você, convidado da pesquisa e outra com o pesquisador responsável. Qualquer dúvida sobre os procedimentos e sua participação nessa pesquisa poderá entrar em contato com os pesquisadores por meio dos telefones e endereços eletrônicos, respectivamente: Tamires de Luccas Bueno (14) 99677-2440 e tamireslbueno@gmail.com ; Heitor Marques Honório (14) 98112- 7777 e heitorhonorio@usp.br e, para denúncias e/ou reclamações poderá entrar em contato com Comitê de Ética em Pesquisa-FOB/USP, à Alameda Dr. Octávio Pinheiro Brisolla, 9-75, Vila Universitária, ou pelo telefone (14)3235-8356, e-mail: cep@fob.usp.br, e a forma de contato com CONEP (endereço, telefone, e-mail), quando pertinente.

Pelo presente instrumento que atende às exigências legais, o Sr. (a) _____, portador da cédula de identidade _____, após leitura minuciosa das informações constantes neste TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO, devidamente explicada pelos profissionais em seus mínimos detalhes, ciente dos serviços e procedimentos aos quais será submetido, não restando quaisquer dúvidas a respeito do lido e explicado, DECLARA e FIRMA seu CONSENTIMENTO LIVRE E ESCLARECIDO concordando em participar da pesquisa proposta. Fica claro que o participante da pesquisa, pode a qualquer momento retirar seu CONSENTIMENTO LIVRE E ESCLARECIDO e deixar de participar desta pesquisa e ciente de que todas as informações prestadas tornar-se-ão confidenciais e guardadas por força de sigilo profissional (Art. 9º do Código de Ética Odontológica).

Por fim, como pesquisador(a) responsável pela pesquisa, DECLARO o cumprimento do disposto na Resolução CNS nº 466 de 2012, contidos nos itens IV.3, item IV.5.a e item IV.6b na íntegra com a resolução CNS nº 466 de dezembro de 2012.

Por estarmos de acordo com o presente termo o firmamos em duas vias igualmente válidas (uma via para o participante da pesquisa e outra para o pesquisador) que serão rubricadas em todas as suas páginas e assinadas ao seu término, conforme o disposto pela Resolução CNS nº 466 de 2012, itens IV.3.f e IV.5.d.

Rubrica do Pesquisador Responsável:

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Bauru, SP, ___ de _____de 2017.

_____ _____

Assinatura do Participante da Pesquisa

Tamires de Luccas Bueno
Assinatura do Pesquisadora
Responsável

O **Comitê de Ética em Pesquisa – CEP**, organizado e criado pela **FOB-USP**, em 29/06/98 (**Portaria GD/0698/FOB**), previsto no item VII da Resolução CNS nº 466/12 do Conselho Nacional de Saúde do Ministério da Saúde (publicada no DOU de 13/06/2013), é um Colegiado interdisciplinar e independente, de relevância pública, de caráter consultivo, deliberativo e educativo, criado para defender os interesses dos participantes da pesquisa em sua integridade e dignidade e para contribuir no desenvolvimento da pesquisa dentro de padrões éticos.

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Alameda Dr. Octávio Pinheiro Brisolla, 9-75
Vila Universitária – Bauru – SP – CEP 17012-901
Telefone/FAX(14)3235-8356
e-mail: cep@fob.usp.br

Rubrica do Pesquisador Responsável:

Rubrica do Participante da Pesquisa :

Annex

ANNEX A- GUIDELINES FOR AUTHORS USED IN PREPARATION OF PAPER 1 AND 2- DENTAL MATERIALS

Introduction

Authors are requested to submit their original manuscript and figures via the online submission and editorial system for *Dental Materials*. Using this online system, authors may submit manuscripts and track their progress through the system to publication. Reviewers can download manuscripts and submit their opinions to the editor. Editors can manage the whole submission/review/revise/publish process. Please register at: https://www.evise.com/profile/api/navigate/DEMA.
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