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THAMIRES PRISCILA CAVAZANA

Efeito do trimetafosfato de sódio, associado ou não ao fluoreto, na biomassa e fluido do biofilme misto contendo *Streptococcus mutans* e *Candida albicans*

Araçatuba

2018

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Dissertação apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba, para obtenção do título de Mestre em Ciência Odontológica, área de concentração Saúde Bucal da Criança.

Orientador: Prof. Titular Alberto Carlos Botazzo Delbem

Coorientador: Prof. Adjunto Juliano Pelim Pessan, Prof. Dr. Douglas Roberto Monteiro

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RESUMO

O presente estudo teve por objetivo verificar o efeito do trimetafosfato de sódio (TMP), associado ou não ao fluoreto (F), sobre células cultiváveis, biomassa total, atividade metabólica e composição da matriz extracelular de biofilmes mistos de *S. mutans* e *C. albicans*, bem como sobre as concentrações de F, cálcio (Ca) e fósforo (P) (biofilme total e fluido do biofilme) e no pH destes biofilmes formados *in vitro*. Para ambos os estudos, os biofilmes foram formados em poços de placas de microtitulação, colocando uma suspenção ($1x10^7$ células/mL *C. albicans* + $1x10^8$ células/mL *S. mutans*) em saliva artificial suplementada com sacarose (0,4%), a qual tinha metade de seu conteúdo renovada a cada 24 horas. Os biofilmes foram tratados três vezes (72, 78 e 96 horas de formação), por um minuto, com soluções contendo TMP (0,25, 0,5 ou 1%) com ou sem 500 ppm F, além de soluções contendo 500 e 1100 ppm F, adotadas como controles positivos. A saliva artificial foi utilizada como tratamento e considerada como controle negativo. Para o estudo mircrobiológico, após o terceiro tratamento foram realizados os testes de quantificação de células cultiváveis (CFU), biomassa total (teste colorimétrico de cristal violeta – CV), atividade metabólica (redução de XTT) e quantificação dos componentes da matriz extracelular (proteína, carboidrato e DNA). Todos os ensaios foram realizados em triplicata, em três ocasiões diferentes. Os resultados foram submetidos à análise de variância a um critério, seguida pelo teste Fisher LSD ($p<0,05$). O TMP apresentou efeito redutor principalmente no metabolismo e nos componentes da matriz extracelular do biofilme. Para o estudo da concentração de F, Ca, e P, após o período de tratamento, estes foram analisados no biofilme total e no fluido do biofilme após a mensuração do pH do biofilme. Em outro conjunto de experimentos, após o terceiro tratamento (96 h de formação de biofilme) o biofilme foi exposto, por 3 minutos, à solução de sacarose a 20%. Esta foi removida e, após 1 minuto, analisou-se o pH do meio e as concentrações de F, Ca, e P tanto na biomassa como no fluido do biofilme. Os dados foram submetidos a análise de

variância a dois critérios, seguida pelo teste de Fisher LSD ($p<0,05$). O tratamento com TMP aumentou a concentração de F e P no fluido do biofilme, além de manter o pH do meio mais próximo do neutro, mesmo após a exposição do biofilme à sacarose. Assim, é possível concluir que o TMP interfere no metabolismo, composição orgânica e inorgânica, bem como no pH do biofilme testado.

Palavras-chaves: Fosfatos, Flúor, Biofilme, *Streptococcus mutans* e *Candida albicans*

Cavazana, TP. Effect of sodium trimetaphosphate, associated or not to fluoride, on biomass and fluid of mixed biofilm containing *Streptococcus mutans* e *Candida albicans*. 2018. 82 f. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2018.

ABSTRACT

The aim of the present study was to verify the effect of sodium trimetaphosphate (TMP), associated or not to fluoride (F), on cultivable cells, total biomass, metabolic activity and composition of the extracellular matrix of mixed biofilms of *S. mutans* and *C. albicans*, as well as on the concentrations of F, calcium (Ca) and phosphorus (P) (total biofilm and biofilm fluid) and pH of these biofilms formed *in vitro*. For both studies, the biofilms were formed in wells of microtiter plates by placing a suspension (1×10^7 cells/mL *C. albicans* + $1x10^8$ cells/mL *S. mutans*) in artificial saliva supplemented with sucrose (0,4%), which had half of its content renewed every 24 hours. Biofilms were treated three times (72, 78 and 96 hours of formation), for one minute, with solutions containing TMP (0.25, 0.5 or 1%) with or without 500 ppm F, as well as solutions containing 500 and 1100 ppm F, adopted as positive controls. Artificial saliva was used as treatment and considered as the negative control. For the microbiological study, the following tests were performed: quantification of cultivable cells (CFU), total biomass (colorimetric crystal violet test - CV), metabolic activity (XTT reduction) and quantification of matrix components (protein, carbohydrate and DNA). All assays were performed in triplicate on three different occasions. The results were submitted to one-way analysis of variance, followed by the Fisher LSD's test ($p<0.05$). TMP showed a reducing effect mainly on the metabolism and components of the extracellular matrix of the biofilm. For the study of the concentrations of F, Ca, and P, these ions were analyzed in the total biofilm and in the biofilm fluid after treatment with the test solutions and after the pH measurement of the biofilm. In another set of experiments, after the third treatment (96 h of biofilm formation), the biofilms were exposed for 3 minutes to a 20% sucrose solution. This was removed and after 1 minute the biofilms were collected, and the pH of the medium and F, Ca, and P concentrations were determined both in the biomass and in the biofilm fluid. The data were submitted by two-way analysis of

variance, followed by Fisher LSD's test ($p<0.05$). Treatment with TMP increased F and P concentration of the biofilm fluid, and maintained the pH of the medium close to neutral values even after exposure of the biofilm to sucrose. Thus, it is possible to conclude that TMP interferes with the metabolism, organic and inorganic composition and the pH of the biofilm tested.

Keywords: Phosphates, Fluoride, Biofilm, *Streptococcus mutans* and *Candida albicans.*

LISTA DE FIGURAS

Capítulo 1

Capítulo 2

LISTA DE TABELAS

Capítulo 1

Capítulo 2

LISTA DE ABREVIATURAS

a.m.	Ante Meridiem
Abs	Absorbance
ANOVA	Análise de Variância/Analysis of Variance
ATCC	American Type Culture Collection
BCA	Bicinchoninic acid
BHI	Brain Heart Infusion
Ca	Calcium
$CaCl_2$	Calcium chloride
CaF_2	Calcium Fluoride
$CaHPO_4^0$	Dicalcium phosphate
CFU	Colony-forming units
cm	centimeter
cm^2	square centimeter
CO_2	Carbon dioxide
CV	Crystal Violet
DNA	Deoxyribonucleic acid
DS	Degree of saturation
EPS	Extracellular polymeric substances
F	Fluoreto
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
Fig	Figure
g	Gramme
h	Hour
HA	Hydroxyapatite
HCl	Hydrochloric acid
HF	Hydrofluoric acid
HPO_4^{2-}	Hydrogen phosphate
IA	Ionic Activities
KCl	Potassium Chloride
l	Liter
Log_{10}	Logarithm to the base 10
mg	Milligram

Mg	Magnesium
min	Minute
ml	Milliliter
mmol L^{-1}	Millimolar
mV	Milivolt
NaCl	Sodium Chloride
NaF	Sodium Fluoride
NaOH	Sodium hydroxide
NC	Negative Control
nm	Nanometer
p	Probability
P	Phosphorus
p.m.	Post meridiem
pH	Hydrogen potential
ppm	Parts per million
rpm	Revolutions per Minute
s	Second
SD	Standard Deviation
TISAB	Total Ionic Strength Adjustment Buffer
TMP	Sodium Trimetaphosphate
UNESP	Universidade Estadual Paulista
UNOESTE	Univerdidade do Oeste Paulista
XTT	(2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5Carboxanilide)
µ	Micro
µg	Microgramme
µl	Microliter
°C	Graus Celsius
g	Gravity

SUMÁRIO

1- Introdução Geral

INTRODUÇÃO GERAL

A cárie dentária é uma doença multifatorial, biofilme-sacarose dependente (Sheiham e James, 2015), ocasionada pela produção bacteriana de ácidos a partir de carboidratos fermentáveis da dieta, a qual progressivamente desmineraliza a estrutura dentária (Cummins e Bowen, 2006). Um dos principais agentes etiológicos da cárie dentária é a bactéria gram positiva *Streptococcus mutans,* devido à sua capacidade de colonizar a superfície dental, metabolizar carboidratos e produzir ácido láctico, além de ter a capacidade de crescer e se multiplicar em ambiente ácido (Marsh e Martin, 2009; Lamont et al., 2006).

O processo de formação de biofilme por esta bactéria inicia-se com o revestimento da superfície do dente pela película adquirida (Bowen e Koo, 2011; Zijnge et al*.,* 2010). A colonização da superfície dentária por *S. mutans* é convencionalmente subdividida em uma fase sacarose independente e uma dependente de sacarose. No biofilme, os microrganismos apresentam-se embebidos em uma matriz extracelular composta por glicoproteínas e polissacarídeos (ten Cate et al., 2009). Inicialmente, várias adesinas de bactérias odontopatogênicas interagem com as glicoproteínas salivares da película adquirida na superfície dos dentes, por meio de ligação a cátions bivalentes. Na presença de sacarose, as bactérias aderem-se firmemente à superfície do dente como resultado da produção de exopolissacáridos (glucanos), por meio da atividade da enzima glicosiltransferase (GTFS). Sendo assim, o acúmulo de biofilme faz com que o *Streptococcus mutans* metabolize eficientemente a sacarose (açúcar ou polímeros, tais como o amido) para produzir grandes quantidades de ácido láctico, capaz de solubilizar o componente mineral do dente e iniciar o processo de cárie (Marsh e Martin, 2009; Lamont et al., 2006).

Além de bactérias, o biofilme dental é composto por vários outros microrganismos, dentre os quais *Candida albicans*, fungo mais comumente encontrado na cavidade oral, que pode ser um fator de risco para o desenvolvimento de cárie dentária (Nikawa et al., 2003). A presença de *C. albicans* é importante na cárie da infância, uma vez que contribui para a patogênese em crianças cárie-ativas (de Carvalho et al., 2006, Klinke et al., 2009). A *Candida* contribui particularmente

para as cavidades dentinárias, por possuir enzimas proteolíticas que realizam a degradação do colágeno (Pereira et al., 2017).

No início do século XXI observou-se um declínio expressivo na prevalência da cárie dentária na maioria dos países desenvolvidos e em desenvolvimento, incluindo o Brasil (Ministério da saúde, Brasil, 2004), sendo que os dentifrícios fluoretados têm contribuído substancialmente para esta redução (Pessan et al., 2011). A utilização dos dentifrícios contendo fluoreto (F) associada à escovação dos dentes é considerada o melhor método preventivo da cárie dentária, visto que associa a remoção ou desorganização periódica do biofilme dental com as propriedades cariostáticas do F (Pessan et al., 2006; Tenuta et al., 2009). Sendo assim, a manutenção da concentração de F na saliva é responsável por seu efeito preventivo e terapêutico, devido ao uso frequente dos dentifrícios. Em acréscimo, a formação de produtos da reação esmalte dentina com F, formando o mineral fluoreto de cálcio (CaF_2), também é responsável pelos efeitos supracitados, uma vez que o depósito destes reservatórios no biofilme dental e em lesões de cárie iniciais é capaz de interferir na progressão da mesma (Buzalaf et al., 2011).

Além do F, as concentrações de fosforo (P) e cálcio (Ca) não somente no biofilme total, mas também no fluido do biofilme, exercem papel fundamental nos processos de des- e remineralização da estrutura dentária. A presença desses íons no ambiente bucal durante o desafio cariogênico proporciona uma diminuição no processo de desmineralização e um aumento no processo de remineralização do esmalte dentário. O processo de remineralização pode ocorrer de duas maneiras: através da precipitação de fosfatos de cálcio ou pelo crescimento dos cristais de esmalte remanescentes através do Ca e P presentes na saliva (Buzalaf et al., 2011). Em acréscimo, a concentração de íons F, Ca e P no biofilme dental apresenta uma relação inversa com a incidência de cárie (Shaw et al., 1983), possivelmente devido à liberação destes íons para o fluido do biofilme, causando uma redução na desmineralização e aumento da remineralização pela supersaturação em relação ao esmalte dentário (Buzalaf et al., 2011).

Concomitantemente ao declínio da carie dentária, tem sido observado um aumento na prevalência de fluorose dentária, sendo a ingestão de dentifrícios fluoretados, especialmente por crianças menores de 6 anos de idade, considerada como o principal fator contribuinte (Warren e Levy, 1999; Mascarenhas, 2000; Wong et al., 2011). Uma alternativa para se minimizar a ocorrência de fluorose é a

diminuição da concentração de F em dentifrícios, embora haja evidência de que tais produtos sejam menos eficazes em crianças com alta atividade de cárie (Lima et al., 2008). Por outro lado, a suplementação com sais de fosfato tem sido uma possibilidade para aumentar a efetividade do F. Estudos *in vitro* e *in situ* demonstraram que dentifrícios com concentração reduzida de F suplementados com trimetafosfato de sódio (TMP) apresentam efeito semelhante à de um dentifrício convencional (1.100 ppm F) sobre a desmineralização do esmalte (Takeshita et al., 2009; Takeshita et al., 2015). Entretanto, os efeitos da associação do TMP e F dependem da proporção entre estes sais (Takeshita et al., 2009; Manarelli et al., 2014) e, assim, para 500 ppm F a concentração ideal de TMP a ser utilizada é de 1% (Takeshita et al., 2011; Takeshita et al., 2015). Em acréscimo, o TMP aumenta significativamente a porcentagem de remineralização de lesões de cárie artificiais (Danelon et al., 2015), uma vez que o efeito deste fosfato está associado à sua capacidade de se adsorver ao esmalte (McGaughey e Stowell, 1977; van Dijk et al., 1980), bem como à retenção de fluoreto de cálcio em suas moléculas previamente aderidas ao esmalte (Danelon et al., 2014; Manarelli et al., 2014). Tais achados foram recentemente confirmados em um estudo clínico randomizado controlado, no qual a progressão de cárie em dentes decíduos foi significativamente menor em crianças que utilizaram um dentifrício contendo 500 ppm F e TMP em comparação a crianças utilizando uma formulação convencional contendo 1100 ppm F (Freire et al., 2016).

Em relação aos efeitos do TMP sobre o biofilme dental, embora a associação entre 500 ppm F e TMP tenha elevado as concentrações de F e Ca no biofilme total a níveis semelhantes aos obtidos quando do uso de um dentifrício convencional (Takeshita et al., 2015), tais aumentos não foram refletidos nas concentrações de F e Ca no fluido do biofilme formado *in situ,* sob desafio cariogênico (Nagata et al., 2017). Com base no exposto, torna-se evidente que enquanto o efeito do TMP sobre o esmalte dentário apresenta grande corpo de evidência científica, os dados sobre os efeitos da associação F-TMP sobre o biofilme dental ainda são escassos e conflitantes. Este aspecto reforça a necessidade de estudos adicionais avaliando os efeitos do F e do TMP sobre o biofilme, especialmente envolvendo métodos analíticos complementares aos utilizados nos estudos supracitados, para uma melhor compreensão dos mecanismos de ação destes íons sobre a dinâmica da cárie dentária.

Portanto, seria interessante conduzir um estudo *in vitro* avaliando os efeitos da associação entre F e TMP sobre a composição e metabolismo de um biofilme misto de *S. mutans* e *C. albicans,* sobre a retenção de F, P e Ca não somente no biofilme total, mas também no fluido do biofilme antes e após a exposição deste à sacarose e sobre o pH deste biofilme.

Para abordar o tema proposto, o estudo será apresentado em dois capítulos distintos, conforme descrito abaixo:

- Capítulo 1: **"Activity of sodium trimetaphosphate, associated or not to fluoride, on dual-species biofilms of *Streptococcus mutans* and *Candida albicans*"**
(artigo preparado para a submissão ao periódico Future Microbiology);

- Capítulo 2: **"Effect of sodium trimetaphosphate, associated or not to fluoride, on the composition and pH of mixed biofilms, before and after exposure to sucrose"**
(artigo preparado para submissão ao periódico Caries Research).

**As referências estão no ANEXO A*

ANEXO A

REFERÊNCIAS:

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ANEXO B

Future Microbiology

Future Medicine - Preparing your article

www.futuremedicine.com/pb-assets/Future%20Medicine%20Author%20Guidelines.pdf

Article sections

The following list provides notes on the key article sections; authors should consult the 'at-a-glance formatting checklist' to determine which sections are required for their submission.

Title

Concisely and clearly conveys the scope/novelty of the article; not more than 120 characters.

Author(s) names & affiliations

Including full name, address and e-mail.

Guidance on author sequence: Author sequence is at the authors' discretion; however, Future Medicine journals suggest following the recommendations in GPP3 Appendix Table 2 (http://www.ismpp.org/gpp3), whereby authors are listed either in order of the level of their contribution, or alphabetically. The corresponding author should always be indicated.

Guidance on a change of affiliation during writing: Where an author has changed their affiliation prior to the publication of an article, the affiliation should reflect where the major part of the work was completed. Current affiliation and contact information should be listed in an acknowledgement.

Abstract

Not more than 120 words; no references should be cited in the abstract. The abstract should highlight the importance of the field under discussion within the journal's scope, and clearly define the parameters of the article.

Structured abstract (for Research articles)

Not more than 120 words, broken down into Aims, Patients & Methods/Materials & Methods, Results and Conclusions. For authors presenting the results of clinical trials, the guidelines recommended by CONSORT should be followed when writing the abstract (http://www.consortstatement.org/), and the clinical trial registration number included at the end of the abstract, where available. Data deposition: where data have been deposited in a public repository, authors should state at the end of the abstract the data set name, repository name and number.

Keywords

Up to 10 keywords (including therapeutic area, mechanism[s] of action etc.) plus names of drugs and compounds mentioned in the text.

Body of the article

The article content should be arranged under relevant headings and subheadings to assist the reader.

Future perspective

The author is challenged to include speculative viewpoint on how the field will have evolved 5– 10 years from the point at which the article was written.

Executive summary

A series of bulleted summary points that illustrate the main topics or conclusions made under each of the main headings of the article.

Summary points (Research articles & Company profiles only)

8–10 bullet point sentences highlighting the key points of the article.

Financial disclosure/Acknowledgements

Disclosing any financial and/or material support that was received for the research or the creation of the work. Also disclosing any relationships any authors have (personal, academic or financial relationships that could influence their actions) or financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. If writing assistance has been used in the creation of the manuscript, this should also be stated and any sources of funding for such assistance clearly identified.

Ethical conduct of research

For studies involving data relating to human or animal experimental investigations, authors should obtain appropriate institutional review board approval and state this within the article (for those investigators who do not have formal ethics review committees, the principles outlined in the Declaration of Helsinki should be followed). In addition, for investigations involving human subjects, authors should obtain informed consent from the participants involved and include an explanation of how this was obtained in the manuscript.

References

Key points

- Authors should focus on recent papers and papers older than 5 years should not be included except for an over-riding purpose.

- Primary literature references, and any patents or websites, should be numerically listed in the reference section in the order that they occur in the text (including any references that only appear in figures/tables/boxes).

- Information from manuscripts submitted but not accepted should be cited in the text as “unpublished observations” with written permission from the source.

- Avoid citing a “personal communication” unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in the text, with written permission from the source.

- References should be denoted numerically and in sequence in the text, using Arabic numerals placed in square brackets, i.e., [12].

- Quote first six authors’ names. If there are more than six, then quote first three et al.

- Reference annotations: 6–8 references should be highlighted that are of particular significance to the subject under review as “* of interest” or “** of considerable interest”, along with a brief (1–2 line) synopsis.

- The Future Medicine Reference Manager and EndNote styles can be downloaded from our website at: https://www.futuremedicine.com/authorguide

Format

- Author's names should appear without full stops in their initials
- Quote first six authors' names. If there are more than six, then quote first three et al.
- A full stop follows authors' names
- Journal name should be in italics and abbreviated to standard format
- Volume number followed by comma, not bold
- Page number range separated by a hyphen with no spaces, followed by the year in brackets, and then a full stop

Examples

Journal example: Fantl JA, Cardozo L, McClish DK et al. Estrogen therapy in the management of urinary incontinence in postmenopausal women: a meta-analysis. Obstet. Gynecol. 83(1), 12–18 (1994).

Book example: De Groat WC, Booth AM, Yoshimura N. Neurophysiology of micturition and its modification in animal models of human disease. In: The Autonomic Nervous System (Volume 6). Andrews WR (Ed.), Harwood Academic Publishers, London, UK, 227–289 (1993).

Meeting abstract example: Smith AB, Jones CD. Recent progress in the pharmacotherapy of diseases of the lower urinary tract. Presented at: 13th International Symposium on Medicinal Chemistry. Atlanta, GA, USA, 28 November–2 December 1994.

Patent example: Merck Frosst Canada, Inc. WO9714691 (1997). (Use the following formats for patent numbers issued by the World, US and European patent offices, respectively: WO1234567, US1234567, EP-123456-A).

Website example (organization homepage): US Food and Drug Association. www.fda.gov

Website example (specific webpage/document): American Cancer Society. Cancer Facts and Figures 2015 (2015). www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2015/index

Milenkovic M, Russo CA, Elixhauser A. Hospital stays for prostate cancer, 2004. HCUP Statistical Brief #30. Agency for Healthcare Research and Quality, MD, USA (2007). www.hcup-us.ahrq.gov/reports/statbriefs/sb30.pdf

Reference annotations

Papers or of particular interest should be identified using one or two asterisk symbols:

- * = of interest
- ** = of considerable interest

Each of the chosen references should be annotated with a brief sentence explaining why the reference is considered to be of interest/particular interest.

Figures, tables, boxes & supplementary materials (incl. video)

Summary figures, tables and boxes are very useful, and we encourage their use in certain article types (see above section on Article types for details on which articles can include figures/tables/boxes). The author should include illustrations to condense and illustrate the information they wish to convey. Commentary that augments an article and could be viewed

as ‘stand-alone’ should be included in a separate box. An example would be a summary of a particular trial or trial series, a case study summary or a series of terms explained.

Figures, tables and boxes should be numbered consecutively according to the order in which they have been first cited in the text.

Figure/table/box guidelines

- File format: All figures, tables and boxes should be submitted in an editable format. For figures that will be included without editing (i.e., photos, imaging data, etc.) please submit as a .jpeg, .pdf or .tiff. Other figures (i.e., graph/bar charts or complex illustrations) should ideally be provided as Adobe Illustrator files (.ai or .eps) if possible, otherwise as a .jpeg, .pdf or .tiff. Tables/boxes should be provided as Microsoft Word, Microsoft Excel or Adobe Illustrator files, and must be editable. If you are uncertain whether the format of your files is appropriate, please check with the Journal Editor.

- Resolution: Figure resolution should be as high as possible, ideally 300 dpi or higher for a .jpeg. Images that are blurry or illegible in any way will not be accepted.

- Font: If possible, please use Helvetica 8pt.

- Abbreviations: All abbreviations used within Figures/tables/boxes should be defined in the legend (even if previously defined in the body of the manuscript).

- Photomicrograph: Please ensure that scale bars are included in figures where appropriate (i.e., photomicrographs). Symbols, arrows or letters used in photomicrographs should contrast with the background. Please explain internal scale and identify the method of staining in photomicrographs.

Future Medicine is able to offer a number of design services to authors, from polishing an existing figures to creating one from scratch (subject to fees). If you would be interested in learning more about this service, please contact Joanne Walker.

Chemical structures

If possible, please submit structures drawn in ISISDraw or ChemDraw format. However, chemical structures can be redrawn in-house. Please use the following conventions:

- Always indicate stereochemistry where necessary – use the wedge and hash bond convention for chiral centers and mark cis/trans bonds as such.

- Draw small peptides (up to five amino acids) in full; use amino acid abbreviations (Gly, Val, Leu, etc.) for larger peptides.

- Refer to each structure with a number in the text; submit a separate file (i.e., not pasted throughout the text) containing these numbered structures in the original chemical drawing package that you used.

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- not more than three figures from works published by a single publisher for an article, and not more than three figures from works published by a single publisher for a book chapter (and in total not more than thirty figures from a single publisher for re-publication in a book, including a multi-volume book with different authors per chapter).

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Units of measurement

Measurements of length, height, weight and volume should be reported in metric units (meter, kilogram or liter) or their decimal multiples.

Temperatures should be in degrees Celsius.

Blood pressures should be in millimeters of mercury.

Any other units should be reported using the International System of Units (SI) where possible.

Statistics

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to judge its appropriateness for the study and to verify the reported results.

When possible, appropriate indicators of measurement error or uncertainty (such as confidence intervals) should be included.

Please define any statistical terms, abbreviations and symbols used.

ANEXO C

Caries Research

Guidelines for Authors
www.karger.com/cre_guidelines

Aims and Scope

'Caries Research' is an international journal, the aim of which is to promote research in dental caries and related fields through publication of original research and critical evaluation of research findings. The journal will publish papers on the aetiology, pathogenesis, prevention and clinical control or management of dental caries. Papers on health outcomes related to dental caries are also of interest, as are papers on other disorders of dental hard tissues, such as dental erosion. Aspects of caries beyond the stage where the pulp ceases to be vital are outside the scope of the journal. The journal reviews papers dealing with natural products and other bacterial inhibitors against specific criteria, details of which are available from the Editor.
Submission

Manuscripts written in English should be submitted online.

Should you experience problems with your submission, please contact

Prof. David Beighton
(Editor-in-Chef, Caries Research)
Department of Microbiology
The Henry Wellcome Laboratories for Microbiology and Salivary Research
KCL Dental Institute, Floor 17, Guys Tower
London Bridge SE1 9RT (UK)
Tel. +44 2071887465
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cre@karger.com

During the online submission you will be asked to list complete mailing addresses, including e-mail addresses of three potential reviewers for your manuscript.

Copies of any 'in press' papers cited in the manuscript must accompany the submission. Manuscripts reporting on clinical trials must be accompanied by the CONSORT checklist (see below).

Plagiarism Policy

Whether intentional or not, plagiarism is a serious violation. We define plagiarism as a case in which a paper reproduces another work with at least 25% similarity and without citation. If evidence of plagiarism is found before/after acceptance or after publication of the paper, the author will be offered a chance for rebuttal. If the arguments are not found to be satisfactory, the manuscript will be retracted and the author sanctioned from publishing papers for a period to be determined by the responsible Editor(s).

Conditions

All manuscripts are subject to editorial review. Manuscripts are received with the explicit understanding that the data they contain have not previously been published (in any

language) and that they are not under simultaneous consideration by any other publication.

Submission of an article for publication implies the transfer of the copyright from the author to the publisher upon acceptance. Accepted papers become the property of Caries Research and may not be reproduced by any means, in whole or in part, without the written consent of the publisher.

For legal reasons, we must receive your **'Submission Statement'** with your original (hand-written) signature. Please download, print, sign and either fax or scan it to make it legallybinding.

It is the author's responsibility to obtain permission to reproduce illustrations, tables, etc., from other publications. Authors of papers describing research on human subjects are required to state that they have adhered to the Declaration of Helsinki.

Types of Papers

Original papers or Short Communications are reports of original work (including systematic reviews and meta-analyses). Both have the structure outlined below but for Short Communications the abstract should be less than 100 words and the manuscript should not exceed 3 printed pages, equivalent to about 9 manuscript pages (including tables, illustrations and references).

Reviews can have a freer format but should nevertheless commence with a Title page, an Abstract and an Introduction defining the scope. Reviews are not subject to page charges.

Current topics are concise articles that present critical discussion of a topic of current interest, or a fresh look at a problem, and should aim to stimulate discussion.

Letters to the Editor, commenting on recent papers in the journal, are published occasionally, together with a response from the authors of the paper concerned.

Preparation of Manuscripts

Text should be one-and-a-half-spaced, with wide margins. All pages and all lines must be numbered, starting from the title page. A conventional font, such as Times New Roman or Arial, should be used, with a font size of 11 or 12. Avoid using italics except for Linnaean names of organisms and names of genes.

Manuscripts should be prepared as a text file plus separate files for illustrations. The text file should contain the following sequence of sections: Title page; Declaration of interests; Abstract; Introduction; Materials and Methods; Results; Discussion; Acknowledgements; References; Legends; Tables. Each section should start on a new page, except for the body of the paper (Introduction to Acknowledgements), which should be continuous. Lines in the manuscript must be numbered consecutively from the title page until the last page. Submissions which do not conform to these simple guidelines will be returned to the author.

Title page: The first page of each manuscript should show, in order:
 the title, which should be informative but concise;
 the authors' names and initials, without degrees or professional status, followed by their institutes;
 a short title, maximum length 60 characters and spaces, for use as a running head;
 a list of 3-10 key words;

 the name of the corresponding author and full contact details (postal address, telephone and fax numbers, and e-mail address).

Declaration of Interests: Potential conflicts of interest should be identified for each author or, if there are no such conflicts, this should be stated explicitly. Conflict of interest exists where an author has a personal or financial relationship that might introduce bias or affect their judgement. Examples of situations where conflicts of interest might arise are restrictive conditions in the funding of the research, or if an author or their employer holds patent(s) on a product used in the study, or payment to an investigator from organisations with an interest in the study (including employment, consultancies, honoraria, ownership of shares, travel grant). Investigators should disclose potential conflicts to study participants and should state whether they have done so.

The possible existence of a conflict of interest does not preclude consideration of a manuscript for publication, but the Editor might consider it appropriate to publish the disclosed information along with the paper.

Abstract: The abstract should summarise the contents of the paper in a single paragraph of no more than 250 words (to ensure that the abstract is published in full by on-line services such as PubMed). No attempt should be made to give numerical results in detail. References are not allowed in the abstract.

Introduction: This section should provide a concise summary of the background to the relevant field of research, introduce the specific problem addressed by the study and state the hypotheses to be tested.

Materials and Methods (or Subjects and Methods): All relevant attributes of the material (e.g. tissue, patients or population sample) forming the subject of the research should be provided. Experimental, analytical and statistical methods should be described concisely but in enough detail that others can repeat the work. The name and brief address of the manufacturer or supplier of major equipment should be given.

Statistical methods should be described with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, findings should be quantified and appropriate measures of error or uncertainty (such as confidence intervals) given. Sole reliance on statistical hypothesis testing, such as the use of P values, should be avoided. Details about eligibility criteria for subjects, randomization and the number of observations should be included. The computer software and the statistical methods used should be specified. See Altman et al.: Statistical guidelines for contributors to medical journals [Br Med J 1983;286:1489–93] for further information.

Manuscripts reporting studies on human subjects should include evidence that the research was ethically conducted in accordance with the Declaration of Helsinki (World Medical Association).In particular, there must be a statement in Materials and Methods that the consent of an appropriate ethical committee was obtained prior to the start of the study, and that subjects were volunteers who had given informed, written consent.

Information detailing the power and sample size calculations must be included in the manuscript.

Randomized clinical trials should be reported according to the standardised protocol of the CONSORT Statement. The CONSORT checklist must be submitted together with papers reporting clinical trials.

Randomized clinical trials must be registered at clinicaltrials.gov or similar national authority and the trial number included in the manuscript.

Trials beginning after 1 July 2012 must be registered before recruitment of the first patient. Caries Research will accept 'retrospective registration' of trials that began before 1 July 2012 (retrospective meaning registration occurs after patient enrolment begins). When submitting a paper on a clinical trial, the trial registration number should be stated at the end of the abstract in the following format: Trial registration: [name of the trial registry, the registry URL and the trial registration number].

In studies on laboratory animals, the experimental procedures should conform to the principles laid down in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and/or the National Research Council Guide for the Care and Use of Laboratory Animals.

Unless the purpose of a paper is to compare specific systems or products, commercial names of clinical and scientific equipment or techniques should only be cited, as appropriate, in the 'Materials and Methods' or 'Acknowledgements' sections. Elsewhere in the manuscript generic terms should be used.

In any manuscript involving microradiography, the following information must be included: the radiation source and filters used and the kV used (this determines the wavelength of radiation and hence the validity of using Angmar's equation).

Manuscripts on experimental enamel caries should show that the lesions retain a relatively well-preserved surface layer, i.e. are not surfacesoftened lesions. Proof of surface integrity can be provided either as illustrations in the paper or as supplementary material for the reviewers. Transverse microradiography, polarized light microscopy of a section immersed in water or backscattered scanning electron microscopy of a polished cross-section can be used to provide the necessary proof. To allow the nature of experimental changes to be assessed, microradiographs or micrographs should be provided to show part of the experimental lesion and the adjacent control (e.g. figure 2 of Zaura et al.: Caries Res 2007;41:489–492). Again, these images can be provided as part of the paper or as supplementary material for review purposes.

Results: Results should be presented without interpretation. The same data should not be presented in both tables and figures. The text should not repeat numerical data provided in tables or figures but should indicate the most important results and describe relevant trends and patterns.

Discussion: This section has the functions of describing any limitations of material or methods, of interpreting the data and of drawing inferences about the contribution of the study to the wider field of research. There should be no repetition of preceding sections, e.g. reiteration of results or the aim of the research. The discussion should end with a few sentences summarising the conclusions of the study. However, there should not be a separate 'Conclusions' section.

Acknowledgements: Acknowledge the contribution of colleagues (for technical assistance, statistical advice, critical comment etc.) and provide the position(s) of author(s) employed by commercial firms. This section should describe the source(s) of funding that have supported the work inlcuding relevant grant numbers. Please also include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript." If this statement is not correct, you must describe the role of any sponsors or funders, and amend the sentence as needed. Additionally, the roles of all authors must be described (For example: Conceived and designed the experiments: AA, BB.

Performed the clinical examination: AA, CC. Performed the experiments: DD, FF. Analyzed the data: BB, FF. Wrote the paper: AA, CC, FF, EE).

Legends: The table headings should be listed first, followed by the legends for the illustrations.

Tables: Tables should be numbered in Arabic numerals. Each table should be placed on a separate page. Tables should not be constructed using tabs but by utilising the table facilities of the word-processing software.

Illustrations:
 Illustrations should be numbered in Arabic numerals in the sequence of citation. Figure numbers must be clearly indicated on the figures themselves, outside the image area.
 Black and white half-tone illustrations must have a final resolution of 300 dpi after scaling, line drawings one of 800-1200 dpi.
 Figures with a screen background should not be submitted.
 When possible, group several illustrations in one block for reproduction (max. size 180 x 223 mm).

Color Illustrations

Online edition: Color illustrations are reproduced free of charge. In the print version, the illustrations are reproduced in black and white. Please avoid referring to the colors in the text and figure legends.

Print edition: Up to 6 color illustrations per page can be integrated within the text at CHF 800.00 per page.

References

Reference to other publications should give due acknowledgement to previous work; provide the reader with accurate and up-to-date guidance on the field of research under discussion; and provide evidence to support lines of argument. Authors should select references carefully to fulfil these aims without attempting to be comprehensive.

Cited work should already be published or officially accepted for publication. Material submitted for publication but not yet accepted should be cited as 'unpublished results', while unpublished observations communicated to the authors by another should be cited as 'personal communication', with credit in both cases being given to the source of the information. Neither unpublished nor personally communicated material should be included in the list of references. Abstracts more than 2 years old and theses should not be cited without a good reason, which should be explained in the covering letter accompanying the paper.

References should be cited by naming the author(s) and year. Where references are cited in parenthesis, both names and date are enclosed in square brackets. Where the author is the subject or object of the sentence, only the year is enclosed in brackets.

One author: [Frostell, 1984] or Frostell [1984].
Two authors: [Dawes and ten Cate, 1990] or Dawes and ten Cate [1990].
More than two authors: [Trahan et al., 1985] or Trahan et al. [1985].

Several references cited in parenthesis should be in date order and separated by semi-colons: [Frostell, 1984; Trahan et al., 1985; Dawes and ten Cate, 1990].

Material published on the World Wide Web should be cited like a reference to a print publication, and the URL included in the reference list (not in the text), together with the year when it was accessed.

The reference list should include all the publications cited in the text, and only those publications. References, formatted as in the examples below, should be arranged in strict alphabetical order. All authors should be listed. For papers by the same authors, references should be listed according to year. Papers published by the same authors in the same year should be distinguished by the letters a, b, c, ... immediately following the year, in both the text citation and the reference list. For abbreviation of journal names, use the Index Medicus system. For journals, provide only the year, volume number and inclusive page numbers.

Examples
(a) Papers published in periodicals: Lussi A, Longbottom C, Gygax M, Braig F: Influence of professional cleaning and drying of occlusal surfaces on laser fluorescence in vivo. Caries Res 2005;39:284-286.

(b) Papers published only with DOI numbers: Theoharides TC, Boucher W, Spear K: Serum interleukin-6 reflects disease severity and osteoporosis in mastocytosis patients. Int Arch Allergy Immunol DOI: 10.1159/000063858.

(c) Monographs: Matthews DE, Farewell VT: Using and Understanding Medical Statistics. Basel, Karger, 1985.

(d) Edited books: DuBois RN: Cyclooxygenase-2 and colorectal cancer; in Dannenberg AJ, DuBois RN (eds): COX-2. Prog Exp Tum Res. Basel, Karger, 2003, vol 37, pp 124-137.

(e) Patents: Diggens AA, Ross JW: Determining ionic species electrochemically. UK Patent Application GB 2 064 131 A, 1980.

(f) World Wide Web: Chaplin M: Water structure and behavior. www.lsbu.ac.uk/water, 2004.

Supplementary Material

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