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COMPILATION OF RESEARCH ABSTRACTS

The *ultra*DEX® (formerly known as RetarDEX*/RetarDENT*) range is backed by extensive research. Please find enclosed abstracts from research papers published in Dental Journals or presented at IADR and AADR meetings, ascertaining to the efficacy of the active ingredients and product formulations.

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The Relationship between Oral Malodour, Gingivitis and Periodontitis/ A Review

Perry A. Ratcliff (DDS) and Paul W. Johnson, Division of Periodontology, Department of Stomatology, School of Dentistry, University of California, San Francisco, CA
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Volatile Sulphur compounds (VSC) are a family of gases, which are primarily responsible for halitosis, a condition in which objectionable odours are present in the mouth air. Although most patients perceive this condition primarily as a cosmetic problem, an increasing volume of evidence is demonstrating that extremely low concentrations of many compounds are highly toxic to tissues. VSC may, therefore, play a role in the pathogenesis of inflammatory conditions such as periodontitis. Since the compounds result from bacterial putrefaction of protein, investigations have been conducted to determine whether specific bacteria are associated with odour production.

Two members of this family hydrogen sulphide (H_2S) and methyl mercaptan (CH_3SH) are primarily responsible for mouth odour. Although many bacteria produce H_2S, the production of CH_3SH, especially at high levels, is primarily restricted to periodontal pathogens. Direct exposure to either of these metabolites adversely affects proteins synthesis by human gingival fibroblasts in culture. However, H_2S has the greatest effect. Other in vitro experiments have demonstrated that cells exposed to CH_3SH synthesise less collagen, degrade more collagen and accumulate collagen precursors, which are poorly cross-linked and susceptible to proteolysis. CH_3SH also increases permeability of intact mucosa and stimulates productions of cytokines, which have been associated with periodontal disease. VSC and in particular CH_3SH are therefore capable of inducing deleterious changes in both the extra cellular matrix and the local immune response of periodontal tissues to plaque antigens. This article reviews these data and emphasises the potential importance of the VSC in the transition of periodontal tissues from clinical health to gingivitis and then to periodontitis.

The treatment of oral malodour should not be considered as just cosmetic therapy since the available evidence indicates that many members of the VSC family are toxic to the periodontal tissues even when present in very low concentrations. Traditional procedures of scaling, root planing and the practice of oral hygiene combined with tongue scraping are effective at reducing levels of these compounds in mouth air and are satisfactory for cosmetic treatment. However, oral care products which can demonstrate efficacy at lowering concentration of VSC in periodontal pockets may also be significant adjuncts to periodontal therapy as well as prevention of gingival disease.

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Management of Periodontitis with Oral Care-Products

W. Conrad Chapek (DDS), K. Omer Reed (DDS), Perry A. Ratcliff (DDS), Emeritus Professor University of California at San Francisco School of Dentistry, USA.
Published: Compendium Continuing Education Dent, Volume 15/Number 6 740-746

Chronic inflammatory periodontal disease is associated with bacteria. Gingivitis and periodontitis are still endemic, despite the fact that several anti-bacterial rinses are available for both prescription and over the counter purchases. This article provides a retrospective analysis that documents the therapeutic capabilities of two oral care products in the management of periodontitis. These two products are currently sold with no anti-bacterial claims.

Materials and methods: Patients were studied from the dental hygiene recall practice of two general dentists. A total of 2,085 periodontal pockets were evaluated in 79 periodontitis patients who had on previous visits received scaling, root planing and curettage as needed.

Patients were accepted into the study when at baseline, there was no immediate need for surgical intervention.

The only variance between the baseline evaluation and the subsequent recall was the addition of RetarDENT* toothpaste and RetarDEX* oral rinse. Both of these products contain 0.1% activated Chlorine Dioxide (ClO_2) with a phosphate as a detergent and stabiliser. Patients were instructed to use both products twice a day in place of their regular home care. The toothpaste was used first followed by the oral rinse.

Results: The formulation of RetarDENT* toothpaste and RetarDEX* oral rinse oral care products used twice a day significantly improved periodontal health with a healing of 1,406 of the original 2,085 pockets. A significant percentage of the probe scores (67.42%) were reduced from →4mm to ←3mm in an average of 3.4 months.

Conclusion: The regimen of using RetarDENT* and RetarDEX* oral care products as presented in this study could be an effective aid for the prevention of periodontitis and in the maintenance of recall hygiene patients, including implant patients.

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Reduction of bleeding on Probing with Oral-Care Products

W. Conrad Chapek (DDS), Illinois, K. Omer Reed (DDS), Pheonix Arizona, Perry A. Ratcliff (DDS), University of California School of Dentistry.
Published: Compendium Volume 16/Number 2

Bleeding on probing is an important indicator for detecting the presence of periodontal disease activity. This retrospective study compared the number of bleeding on probing sites that measured 4mm or more with the use vs the non use of RetarDEX* oral rinse and RetarDENT* toothpaste.

Results: At the beginning of the study 11 patients had a record 239 bleeding sites with probe scores of 4mm or more. At the second re-care visit the total number of bleeding sites had increased after the patients had had OH instruction but continued to use their regular family toothpaste and oral rinse. After the second visit patients were given the test products RetarDEX* oral rinse and RetarDENT* toothpaste and asked to use them twice a day. After a mean of 6.9 months between the second and third re-care prophylaxis visits, the hygienists re-scored the mouth, recording all bleeding sites. There were only 72 bleeding sites compared to the original 256 before the test products were used.

Conclusion: This study shows that the twice-daily use of RetarDEX* oral rinse & RetarDENT* toothpaste will reduce bleeding on probing. Mechanisms of action were postulated, however, further research is needed to clarify the nature of processes involved.

Germicidal effect of Povidone-Iodide and Chlorine Dioxide ClO_2 on Dental Pathogens

Perry A. Ratcliff (DDS) and V. Bolin, Northern Arizona University, Flagstaff, Arizona, USA.
Presented to an AADR meeting
Published: J Dent Res.; 71 Sp. Iss:189

Periodontal diseases are associated with different bacteria. The germicidal effect of povidone-iodide and ClO_2 in aqueous solution were studied in vitro against those bacteria believed to be associated with dental disease.

Method: Fresh 48 hour cultures of Streptococci mutans and Antiobacillus actinomycetumitans (Aa) were grown on chocolate agar in a BBL GasPak system. Diluted cultures were added to appropriate dilutions of povidone-iodide or aqueous ClO_2 adjusted to a pH of 5. The anaerobes were diluted through 10-4 and the aerobes 10-5. All cultures incubated 72 hours and colonies counted.

Results: Bactericidal activity of RetarDEX* oral rinse ClO2 /phosphate solution in % kill at pH 6.5

Time (seconds)	Aa	P. gingivalis	S. mutans	A. Sanguis	C. albicans
10	99+	82	94	6	--------
30	99+	84	99	48	97.5
60	99+	94	99	40	99.0+

Conclusion: The high kill ratio of S. mutans suggests the ClO_2 containing rinse would be effective in reducing caries. The high kill ratio of Aa. and P. gingivalis suggests that the ClO_2 containing rinse is formulated to be an effective control agent for both caries and periodontal disease.

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Chlorine Dioxide ClO_2 /Phosphate Germicide vs. Actinobacillus actinomycetemcomitans (Aa) and Prophromonas (P). (Bacteroides) Gingivalis

Perry A. Ratcliff (DDS) and V. Bolin, Northern Arizona University, Flagstaff, Arizona
Presented: Research abstract 669 at the AADR Annual meeting

The purpose of this study was to determine the bactericidal effectiveness of ClO_2/ phosphate solutions against Aa and P. gingivalis.

Method: Using standard anaerobic culture techniques the percentage kill rate of Aa. and P. gingivalis against a ClO_2/ phosphate solution were recorded.

Results: Aa. was killed in 10 seconds by 0.1% ClO_2, pH 6.5, without serum at 99% and with serum at 99%. P. gingivalis was killed in 10 seconds without serum at 99%, but with serum at 82%.

Conclusion: We conclude that ClO_2/phosphate solutions at 0.1% chlorine dioxide concentration, pH 6.5, was an effective germicide against both organisms tested.

Use of an oxidising agent to destroy amino acids to prevent their use as building blocks for protein

Perry A. Ratcliff (DDS)
Presented: IADR meeting Nice, France

Bacteria use amino acids to synthesise proteins. If available amino acids were reduced or eliminated, there should be a reduction in the ability for micro organisms to multiply in body cavities.

Method: 17 essential and non-essential amino acids, in the absence of their transaminases, were placed in 4 vials with verified concentrations of each amino acid (baseline). 0.01% chlorine dioxide solution was added to 3 vials. The forth vial was kept as a control. At the end of 1, 3, and 5 minutes all vials were assayed by iodometric methods after removal of residual chlorine dioxide by thoisulfate. Evaluation of each was by high performance liquid chromatography.

Results: After 5 minutes at baseline the percentage changes of decrease in amino acids were:

Argenin	35.57%	Histidine	49.68%
Isolecine	36.80%	Leucine	21.93%
Lysine	33.33%	Methionine	70.91%
Phenylalanine	35.10%	Threonine	26.23%
Valine	37.50%	Alanine	23.49%
Aspartic Acid	12.08%	Cysteine	99.19%
Glutamic	10.69%	Glycine	67.94%
Proline	6.78%	Serine	44.18%
Tyrosine	27.44%		

Conclusion: In the absence of transaminases a 0.01% solution of chlorine dioxide is a partially effective agent to de-activate all amino acids within 5 minutes using this procedure.

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Anti-microbial capacity of a Chlorine Dioxide based toothpaste

Perry A. Ratcliff (DDS) and V. Bolin
Northern Arizona University, Flagstaff, Arizona, USA
Presented: IADR meeting

The purpose of this study was to determine the percent kill in a simulated oral environment by a dentifrice containing 0.1% chlorine dioxide against Antiobacillus actinomycetumitans (Aa) and Porphyromonas gingivalis.

Method: Ten grams of toothpaste were placed into each of two sterile 50ml beakers, each containing a magnetic stir bar. 10ml sterile distilled water was added to a third beaker as a control. 1.6ml 10% sodium thiosulfate was added to the first beaker to neutralise the chlorine dioxide in the toothpaste.
Subsequently, 18.4ml calf serum was added. 20ml calf serum was added to the remaining two beakers. Toothpaste suspensions were mixed thoroughly on a magnetic mixer. While mixing, 3ml of the test organisms suspension was added. At 10 and 30 second intervals, 10ml was removed from the beaker and placed in a 16 x 125mm tube which contained 2ml 15% sodium thiosulate. Tubes were capped, mixed and anaerobic plate counts performed employing spread method on anaerobic blood agar. Plates were incubated in a candle jar. The experiment was repeated.

Results: Aa and P. gingivalis were killed at the 99%+ level in 10 seconds and 30 seconds with a 0.1% chlorine dioxide formulation in a simulated oral environment.

Conclusion: The chlorine dioxide toothpaste was an effective germicide against both organisms tested.

Effects of Chlorine Dioxide mouthrinse on oral Streptococci, Lactobacilli and Candida Albican

Edward Lynch, D. Gill, S. Wakefield, A. Kersey, and K. Seymour. The London Hospital Medical College, London
Published: J Dent Res. Volume 75(5):1187

It has been claimed that Chlorine Dioxide containing mouth rinses have anti-microbial activity without significant local side effects.

Method: In this clinical trial a 0.1% Chlorine Dioxide mouth rinse (RetarDEX* oral rinse) was tested for its activity against oral mutans streptococci, lactobacilli and candida albicans using standard chair side microbiological diagnostic kits (Vivacare, Liechtenstein). Thirty-three patients (mean age = 62 years) were recruited into the study.

Subjects were requested to rinse for sixty seconds three times daily with the mouth rinse for a period of two weeks. Mutans streptococci, lactobacilli and candida albicans were cultured from salivary samples at baseline and two weeks. Counts of micro organisms in saliva were then recorded.

Results: From this data, there were reductions in counts of both mutans streptococci and lactobacilli ($p \leftarrow 0.01$) but no change was detected with candida. A control group of dental students rinsed with a placebo under the same circumstances and no reduction of counts were noted.

Conclusion: From this clinical trial it would appear that RetarDEX* oral rinse is capable of reducing salivary mutans streptococci and lactobacilli.

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Evaluation of Oral Health Care Products to Alleviate Oral Malodour

Professor Martin Grootveld (BSc PhD MIBMS CBiol FSB FRSC MK), C.J.L Silwood and Edward Lynch
Inflammation Research Group and Department of Conservative Dentistry, St Batholomew's and the Royal London School of Medicine and Dentistry, London
Presented: IADR Orlando, USA

Oral Malodour (Halitosis) is generally ascribable to oral microbial putrefaction generating malodourous volatile sulphur compounds (VSCs) which predominantly comprise dihydrogen sulphide and methyl mercaptan. This study assesses the relative effectiveness of 6 oral hygiene products. (OHCP's) [1-6] in reducing oral cavity VSCs.

Method: A mixed model 3-factor factorial experimental design involving 6 volunteers, 7 treatment regimens (products [1-6] and water placebo) and 5 time-points (0.00-3.31) was undertaken. Electron-donating VSC levels were determined in triplicate using sulphide monitor (Interscan model 1170) both prior to (0.00hr.) and following oral rinsing (20ml) or chewing (2 capsules of [6]) episodes with each product examined (0.30mins, 1.30, 2.30 and 3.30hr. post administration).

Results: The findings were recorded as peak and steady-state VSC equivalents (p.p.b.). With the exception of product [6], each OHCP tested was found to reproducibly reduce VSC concentrations within 20 minutes of treatment.

Conclusion: The most effective OHCP's ([1-3]) contain admixtures of chlorite anion and chlorine dioxide. (Both of these agents have the ability to directly oxidise VSC to non-malodourous products and the latter is powerfully cidal towards odourigenic micro-organisms).
[1] RetarDEX* oral rinse, [2] Profresh, [3] Oxyfresh, [4] Listerine Mint, [5] Scope, [6] Breath Assure Capsule.

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Long-term diurnal investigation of the capacity of an oral healthcare product to neutralise Oral Malodour

A Report Prepared by Professor Martin Grootveld (BSc PhD MIBMS CBiol FSB FRSC MK) Biomedical and Clinical Research Ltd, Atherton, Manchester M46 0DR
Data on file

In this investigation, the clinical effectiveness of an oral rinse product (UltraDEX® Daily Oral Rinse), tested against a water (H_20) placebo treatment, towards oral malodour (halitosis) was determined using a newly-developed, portable gas-chromatographic system with the ability to determine parts-per-billion (ppb) levels of 3 different volatile sulphur compounds (VSCs), Hydrogen Sulphide, Methyl Mercaptan and Dimethyl Sulphide, in air directly sampled from the oral cavity.

Materials and methods: 30 non-smoking volunteers (17 male, 13 female) ranging in age from 24 to 55 years took part.

The relative effectiveness and longevity (up to 12.16* hr. post-administration) of the above commercially-available mouth rinse in suppressing oral malodour was explored. VSC levels in air were directly sampled from the oral cavity of the participants before and at pre selected time points following the administration or either mouth rinse (UltraDEX and Water). An OralChroma™ portable gas chromatographic device was used for the simultaneous and rapid determination of 3 different VSC (Hydrogen Sulphide, Methyl Mercaptan and Dimethyl Sulphide) in the oral cavity at each sampling time-point.

A 1.00 ml volume of air was extracted and exactly 0.50 ml of each sample was injected into the OralChroma™ device.

Results were recorded as parts-per-billion (ppb) oral cavity concentrations for each of the VSC, at each time point; 0.00hr baseline and again at 0.33, 4.00, 8.00, and 12.00 hr post-administration.

Results: Application of the UltraDEX® oral rinse formulation as a treatment for oral malodour gave rise to extremely highly significant differences between the mean oral cavity Hydrogen Sulphide and Methyl Mercaptan (H_2S and CH_3SH respectively) concentrations between the 0.00 hr. (pre-treatment) time-point and those at 0.33, 4.00, 8.00 and 12.00 hr. post-treatment ($p \leftarrow 0.0001$ in each case), specifically substantial reductions in their post-treatment oral cavity concentrations. For the VSC Dimethyl Sulphide ($(CH_3)2S$), there were also highly significant time-dependent decreases in the mean 0.00 hr. level of this VSC, specifically those observed at the 0.33 and 12.00 hr. time-points ($p = 0.001$ and 0.002 respectively).

For the water placebo treatment, significant differences were only found between the 0.00 and 12.00 hr. time-point mean values for H_2S ($p \leftarrow 0.0001$); the 0.00 and 4.00 hr, and 0.00 and 12.00 hr. time-point mean values for CH_3SH ($p = 0.003$ and 0.001 respectively); and only the 0.00 and 12.00 hr. time-point mean values for $(CH_3)2S$ ($p = 0.001$).

Conclusion: For H_2S, CH_3SH and CH_3SCH_3, the UltraDEX oral rinse formulation exerted very highly significant VSC-neutralising activities which were of a significantly greater magnitude than those observed with the water placebo control rinse (especially for H_2S).

Therefore, data acquired clearly confirm that the UltraDEX oral rinse product continues to exert its oral malodour-neutralising effects at the 12.16 hr. post-rinsing time-point.

* Each determination took 8 minutes for the gas chromatographic separation of the three different VSCs; the second series of replicate measurements were completed 20 minutes (0.33 hours) later (16 minutes in total for the two chromatographic runs, and 2 x two minute intervals for sample collection and injection). The average time taken between measurements has resulted in a time point of over 12 hours at 12.16hrs.

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Reduction of Volatile Sulphur Compounds (VSCs) after using RetarDEX Oral Rinse

Measurements taken using a Halimeter instrument. Conducted by Analyse Inc; Tempe, Arizona USA
Data on file

This study was conducted to determine the efficacy of a Chlorine Dioxide containing mouthwash (RetarDEX* oral rinse) in reducing VSCs.

Method: An initial measurement of halimeter response to volatile sulphur compounds in a subject's breath was taken before using RetarDEX* oral rinse. Unflavoured RetarDEX* oral rinse was then 'swished' in the mouth and the breath was immediately sampled and again after 5 hours had elapsed.

Results: Within 15-30 seconds, there is approximately 50% reduction in 'breath odour' as a result of rinsing with unflavoured RetarDEX* oral rinse. In the absence of eating or smoking, the instrument response appears to decrease further over a five-hour period.

Conclusion: The significant reduction in VSCs confirms the efficacy of RetarDEX* oral rinse.

Relative Dental Abrasion of Dentifrices

Data courtesy of ADA Conducted by Oral Health Research Institute, Indianapolis, Indiana, USA
Data on file

Dental professionals know low-abrasive toothpastes are especially desirable when patients have exposed root areas or cosmetic restorations such as delicate veneers. Low abrasivity is preferable over the long term just to preserve the current state of our teeth.

Method: 10 top commercial toothpastes were tested following the ADA standard 100 comparison and scored on a scale of 0-225.

Results: RetarDENT* Toothpaste registered 53, the second lowest of the toothpastes studied.

Conclusion: RetarDENT* Toothpaste has a lower level of abrasivity compared to 9 out of the 10 toothpastes tested.

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Odour Reduction Potential of a Chlorine Dioxide containing Mouthrinse

J. Frascella (DMD), R.D Gilbert (PhD), P. Fernandez (RDH), TKL Research Inc. New Jersey
Published: The Journal of Clinical Dentistry, Volume 9/ Number 2

Studies on the aetiology of halitosis indicate that hydrogen sulphide, methyl mercaptan and to a lesser extent, dimethyl sulphide, referred to as volatile sulphur compounds (VSC), are the principal cause of oral malodour. The dorso-posterior surface of the tongue and other aetiologies include poor oral hygiene, periodontal disease and a dry mouth. Comprehensive reviews cover the latest scientific findings in the area of halitosis research. Studies have suggested that chlorine dioxide, when contained in a mouthwash, neutralises VSC in mouth air. Lunch et al have demonstrated, in experimental models, that the amino acids cysteine and methionine (precursors of VSC responsible for oral malodour) are consumed by oxidation. Reed graphically described the chemical process by which chlorine dioxide produces oxygen, which chemically degrades VSC. Thus, with a mouthwash containing chlorine dioxide, oral malodour is reduced because the VSC are reduced. The purpose of this 12-subject study was to determine the effect a commercially available mouthwash, containing chlorine dioxide, on oral malodour in otherwise healthy individuals.

Method: The efficacy of a chlorine dioxide containing mouthwash in reducing oral malodour was compared to that of a water control in a randomised, double-blind crossover study in 12 male and female subjects. Prospective subjects were recruited from a computerised database of individuals already known to the research centre and pre-screened by telephone. All medical conditions such as diabetes were ruled out. Odour pleasantness scores (7-point scale) and odour intensity scores (5-point scale) were analysed using the Analysis of Variance (ANOVA).

Subjects who met the minimum malodour criteria on treatment Day 1 were dispensed 15ml of RetarDEX* oral rinse or the water control in a cup and were, under supervision, asked to vigorously rinse at 30 second intervals. The rinsing time was recorded; subjects underwent further breath assessments at 0.5, 1,2 and 4 hours post rinsing. After completing a wash out period of 96 hours the two groups crossed over to the alternate products on treatment Date 2.

Results: Twenty-two subjects met the selection criteria. Of these 17 subjects returned for hedonic baseline assessment to establish minimum malodour criteria on treatment Day 1. In the chlorine dioxide group, a significant improvement in mouth odour pleasantness was evident at 0.5 hours post rinsing and persisted through the final evaluation time point of 4 hours.

Conclusion: A significant improvement in mouth odour pleasantness compared to the control was evident with the chlorine dioxide mouthwash at the first post-rinsing evaluation (0.5 hours) and persisted through the last post-rinsing evaluation (4 hours). This finding suggests that the chlorine dioxide mouthwash has a rapid onset of action and a lasting effect on oral malodour. It is concluded that a one-time use of a chlorine dioxide containing mouthwash significantly reduces mouth odour unpleasantness and intensity for at least 4 hours.

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Effects of stabilised Chlorine Dioxide and Chlorhexidine Mouthrinses in vitro on cells involved in periodontal healing

M. Robert Wirthlin (DDS), Brand J. AHN (DDS), Belma Enriquez (BS), M. Zamirul Hussain, (PhD)
Published: Periodontal abstract Volume 54/ Number 3

Clinicians may elect to prescribe the use of an antimicrobial mouth rinse as an adjunct to manual oral hygiene procedures and after oral surgical procedures when physical oral hygiene may be limited by surgical dressings, sutures, and tender gingival tissues. Medicated mouth rinses might improve taste, odour and comfort for the patients during the post-surgical period. The problem for the clinician is balancing the need for plaque suppression and a possible detrimental effect on healing tissues, especially for those regenerative procedures.

Discussion: Our study confirms the foregoing reports that Chlorhexidene is toxic to human cells pertinent to periodontal wound healing and includes cultured bone cells not explicitly tested before. There are clinical reports of CHX mouth rinse used post-surgically in humans and dogs for control of plaque that describe good results. However, in cases of regenerative procedures where CHX might wick into subgingival areas wherein CHX could destroy the friable new granulation from the periodontal ligament or bone, there could be a risk. One might not consider using CHX until epithelial attachment seal is formed.

That is usually at 6-7 days of healing in a periodontal flap surgery; but after guided tissue regeneration procedure there may be no seal until the barrier is removed. Stabilised Chlorine Dioxide could be safer on cells during periodontal wound healing. ClO_2 contains no alcohol, does not cause staining of teeth, restorations, or soft tissue, does not cause calculus formation and has not been reported to affect taste or cause an allergic reaction. ClO_2 free radical ions do not allow resistant species to develop.

Conclusion: Stabilised Chlorine Dioxide mouth rinse is less toxic than Chlorhexidene to human gingival fibroblasts, periodontal ligament cells and osteoblast cell line in vitro.

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Multicomponent Spectroscopic investigations of Salivary Antioxidant Consumption by Oral Rinse preparation containing the stable Free Radical Species Chlorine Dioxide (ClO_2)

Edward Lynch, A. Sheerin, A.W.D Claxson, M.D Atherton, C.J Rhodes, C.J.L Silwood, D.P Naughton and Professor Martin Grootveld (BSc PhD MIBMS CBiol FSB FRSC MK)
Dept of Conservative Dentistry & Inflammation Research Group, St Bartholomews & the Royal London Hospitals Schools of Medicine & Dentistry, London. Department of Chemistry, Queen Mary and Westfield College, University of London, London.
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A multicomponent evaluation of the oxidative consumption of salivary biomolecules by a commercially available mouth wash (RetarDEX* oral rinse) preparation containing an admixture of the stable free radical species chlorine dioxide (ClO_2.) with chlorite anion (ClO_2-) has been investigated using high resolution proton (1H) Nuclear Magnetic Resonance (NMR) spectroscopy.

Method: Unstimulated human saliva samples were collected from ten healthy volunteers. To some samples RetarDEX* oral rinse was added. To further samples, doubly-distilled H_2O was added as a control. Aqueous solutions containing 1.00 x 10.2 mol.dm .3 sodium pyruvate, L-cysteine or L-methionine were prepared in a phosphate buffer (pH 7.00). Samples of these solutions were treated with RetarDEX* oral rinse and additional aliquots of each solution treated with equivalent volumes of doubly-distilled H_2O served as controls. The samples were subject to (1) 1 H NMR measurement, (2) spectrophotomeric and (3) ESR (electron spin resonance) analysis.

Conclusion: The results obtained demonstrated that ClO_2. and/or ClO_2- present in this preparation affected the oxidative decarboxylation of salivary pyruvate (to acetate and CO_2). Experiments conducted on chemical model systems confirmed the oxidative decarboxylation of pyruvate by RetarDEX* oral rinse and also demonstrated that the amino acids cysteine and methionine (precursors to volatile sulphur compounds responsible for oral malodour) were oxidatively consumed.

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Periproducts Limited, PO Box 176, Ruislip
Middlesex, HA4 9YR, United Kingdom

Email: dental@periproducts.co.uk
Telephone: +44 (0) 20 8868 1500 Fax: +44 (0) 20 8868 9011

www.periproducts.co.uk