O-37 Determination of ABO blood grouping using tooth material Supporting information for forensic identification

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Tooth is the most robust and stable part of the human body, and therefore potentially very useful for identifying severely burned bodies for forensic purposes. However, conventional dental records may not be available or sufficient for such purpose. To study the efficiency and robustness of ABO blood grouping from tooth material, extracted tooth samples from 146 people were ABO blood grouped by absorption elution technique from enamel, dentine and pulp, with direct blood grouping at the time of extraction as control. Of the 146 tooth samples, a half of 64 teeth without caries and 56 whole teeth with caries were blood grouped immediately. The other half of the 64 teeth without caries were stored at room temperature (29+4°C) for one month.

The results show from enamel, the proportion of correctly ABO blood grouped tooth samples without caries was only 37 to 69% and significantly smaller ($p<0.01$) than from dentine, pulp or control (blood). In comparison, for dentine and pulp 94 to 100% of the results were correct, and there was no significant difference between dentine, pulp and control immediately after extraction. With the exception of relatively unreliable blood grouping from enamel, storing non-caries teeth for one month at room temperature appears to exert no significant influence in comparison with immediate blood grouping after extraction. However, one month underground made it significantly less likely ($p<0.01$ for dentine and pulp) to achieve correct blood grouping from non-caries tooth material in comparison with immediate blood grouping after extraction or one month storage at room temperature. For dentine and pulp, only 65-75% of blood grouping results were correct for teeth with caries. Particularly caries pulps appears to make correct blood grouping from tooth material (dentine and pulp) significantly less likely ($p<0.01$) than from teeth with no caries. Similar tendency for teeth with caries dentine was weaker, but there was no significant difference in correct blood grouping from teeth with caries dentine and caries pulps.

The results confirm that enamel alone is unreliable material for ABO blood grouping. However, dentine, pulp and probably whole teeth without caries can be used for blood grouping with reasonable confidence. The material from a single tooth appears sufficient for blood grouping in such cases. The results also imply adverse effects of microbial contamination by caries and soil contact, which can limit the reliability of correct blood grouping from teeth in forensic applications. When the choice is possible, tooth material with as little caries as possible should be used.

P-1

The effect of rewetting agents on *in vitro* recurrent caries. *Itthagarun A[1]; Tay FR[1]; King NM[1]; Wefel JS[2]; Pashley DH[3] ([1]University of Hong Kong, HKSAR; [2]University of Iowa, USA; [3]Medical College of Georgia, USA)

This study examined the *in vitro* caries inhibiting potential of fluoride (FR) and non-fluoride-containing (NFR) rewetting agents that are applied to acid-etched enamel and dentin before the use of water-free, dentin adhesives. Twelve caries-free bicuspids were divided into three groups. 2 x 3 x 1.5 mm cavities were prepared on the mesial and distal surfaces of each tooth, with half of the cavosurface margin in enamel and half in cementum. In group I (control), One-Step (Bisco, Schamburg, USA) was applied without etching or rewetting agents. In group II, cavities were acid-etched, air-dried for 2s, rewetted with a NFR (Aqua-Prep, Bisco) for 20s, and then bonded with One-Step. Treatment for group III was similar to group II, except that a FR (Aqua-Prep F, Bisco) was used. Bonded cavities were restored with a non-fluoride-containing composite (ÆliteFlo, Bisco). Artificial carious lesions were induced in these specimens, from which 100±20 µm thick longitudinal sections were subsequently prepared; yielding 16 specimens per group for evaluation with polarizing light microscopy and microradiography. Representative sections were processed for transmission electron microscopy (TEM). Undemineralized ultrathin sections were examined unstained. Results: The outer lesion depths (µm) were 116±5, 114±5 and 113±7, and the lesion areas (µm²) were 21,562±2,035, 14,966±1,819, 10,829±2,302 for groups I, II and III respectively. The differences were not statistically significant for lesion depth ($p>0.5$, ANOVA, Duncan's test), but highly significant for lesion area ($p<0.001$). Wall lesions were consistently present in group I, while inhibition zones were invariably observed in group III. 87.5% of group II specimens exhibited neither wall lesions nor inhibition zones. Inhibition zones in Group III had a mean width of 52.80±18µm. TEM showed that remnant dentin crystallites within the inhibition zones in group III were larger and denser than the corresponding wall lesions. They were of the same density and size along the same lesion depth in group II specimens. It is hypothesized that a fluoride-containing rewetting agent inhibits recurrent caries *in vitro* by altering apatite dissolution. (Supported by University of Hong Kong CRC grant 10202354)

P-2

The First Report of *Candida dubliniensis* from Human Root Caries Lesions. S SHEN, H.K. YIP*, L.P. SAMARANAYAKE and J.E. DYSON (Faculty of Dentistry, The University of Hong Kong, Hong Kong BAR, China).

Candida dubliniensis is a newly described fungal species generally isolated from HIV-infected patients and considered an emerging opportunistic oral pathogen. However there are recent reports of its carriage in healthy individuals as well as in non-oral sites. As little is known about the prevalence of *Candida dubliniensis* in root caries lesions, we characterized 29 yeast isolates from root caries lesions for the presence of this potential pathogen. A total of 29 *Candida* isolates were obtained from 19 root caries lesions in elderly ethnic Chinese (12 patients, mean age 81.67 ± 6.30, 3 males and 9 females) as described in our previous communication (Shen *et al. J Dent Res* 2000;79 special issue:395). *Candida* species were identified using the "germ tube" test, API 20C AUX yeast identification kit with an newly updated identification database (bioMerieux sa, Marcy-l'Etoile, France) and growth at 45°C for 48 hrs (Jabra-Rizk *et al. J Clin Microbiol* 1999;37:1464). All yeasts were biotyped using the method of Williamson *et al. (Microbios* 1987;51:195). Among 29 yeast isolates, three were identified as *Candida dubliniensis* (10.34%), two were *Candida glabrata* (6.90%) and the remainder were *Candida albicans 1* (82.76%). The biotypes of all isolates varied considerably. Our study reports, for the first time, the presence of *Candida dubliniensis* in root caries lesions. The presence of this rather virulent breast pathogen in root caries lesions of elderly is disconcerting, as it may cause systemic morbidity in compromised situations. (Supported by a CRCG grant of the University of Hong Kong, Hong Kong BAR, China.)

P-3

Intra- and Inter-species Coaggregation of Bacterial Isolates from Root Surface Caries Lesions. S SHEN*, L.P. SAMARANAYAKE and H.K. YIP (Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China).

Bacterial coaggregation reactions between different species and the autoaggregation of the same species are associated with the initiation and development of dental plaque and biofilms. As no such data is available on bacterial isolates from root caries lesions, we evaluated the coaggregation of 22 different bacterial species comprising 10 different genera, from human root caries lesions. Bacteria were isolated from 30 root caries lesions in elderly Chinese and identified using standard microbiological criteria (Shen *et al. J Dent Res* 2000;79 special issue:395). Intra- and inter-species coaggregation was evaluated both by a qualitative visual scoring system (Cisar *et al. Infect Immun* 1979;24:742) and a quantitative spectrophotometric assay (McIntire *et al. Infect Immun* 1978;21:978). The quantitative coaggregation assay we used proved to be a more sensitive method than the qualitative visual evaluation as the results yielded the percent coaggregation. Inter-species coaggregation was seen between: *1) Actinomyces spp.* and *heillonella* spp.; 2) *A. israelii* and *Peptostreptococcus prevotii;* 3) *Actinomyces spp.* and *Bacteroides gracilis; 4) Bacteroides intermedium* and 9 different species, and 5) *Fusobacterium* spp. and 6 other species. These results imply the existence of multiple interactions between the congregation inducing bacterial species during root caries formation. In particular, *Actinomyces V. Veillonella Bacteroides spp.* and *Fusobacterium* app. appear to play a significant role in this context. (Supported by CRCG grant of the University of Hong Kong, Hong Kong SAR, China.)

P-4

Microbial-flora of root canals at the time of root filling and the outcome of treatment.

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The role of bacteria in the pathogenesis of pulpal and periapical diseases is well documented. The presence of bacteria in the root canal at the time of obturation may have an impact on the outcome of treatment. The presence study investigated the microflora of root canal at the time of root filling and the outcome of treatment. Samples were collected in the student clinic from teeth undergoing root canal treatment. At the obturation appointment, the root canal content was sampled prior to root filling and cultured anaerobically. 31 single rooted teeth were sampled and bacterial growth was detected in 17 teeth (55%). A total of 15 species were recovered, 2 of which were strict anaerobes and the remaining 13 were facultative anaerobic and aerobic organisms. All patients were invited for review in 6 months and 27 (response rate 87%) were examined. 13 teeth (48%) were considered as successful under strict clinical and radiographic criteria. No significant difference was detected among those teeth with or without positive culture at the time of obturation on the outcome of treatment. The result suggested that the presence of bacteria at the time of root filling did not affect the outcome of root canal treatment in the 6 months period. Long-term follow up is required to assess the impact of bacteria on the treatment outcome.

P-5

"COMPARISON BETWEEN GTF 3-D MODELS PROVIDES POSSIBILITY FOR VACCINE DEVELOPMENT" Y.-W. TSAI*, Y.-Y. SHIAU, J.-S. CHIA, H.-C. CHOU, Y.-C. LIAW, K.-L. LOU. (Graduate Institute of Oral Biology, College of Medicine, National Taiwan University, Taipei 100, TAIWAN.)

Glucosyltransferases (GtfB/C/D) of *S. mutans*, a pathogen for human dental caries, synthesize water-insoluble glucan through hydrolysis of sucrose. Genetic and biochemical approaches have identified several active sites of these enzymes, but no three-dimensional structural evidence is yet available to elucidate the subdomain arrangement and molecular mechanism of catalysis. Based on a combined sequence and secondary structure alignment against known crystal structure of segments from closely related proteins, we propose here the 3-D models of the N-terminal domains essential for the sucrose binding and splitting in all three GTFs. Tim-barrel of $(\alpha/\beta)_8$ structural characteristics is revealed and the structural correlation for two peptides Gtf-P1 and Gtf-P2 (active sites) is described. Functional analysis according to the recognition of antibody against Gtf-P1 by reducing the enzymatic activity has also been accomplished. Conclusion: Monoclonal antibody against Gtf-P1, which then influences Gtf-P2, can be good candidates for developing vaccines to prevent human dental caries via disturbing GTF enzyme function. (supported in part by grant NSC 89-2314-B-002-258)

P-6

Cytotoxicity of Fluoride on Human Pulp Cell Cultures *in vitro*. K. W. Tai*, Y C Chang (School of Dentistry, Chung Shan Medical and Dental College, Taichung, Taiwan)

The use of glass-ionomer cements in restorative dentistry has increased considerably, due to their excellent chemical properties. Numerous studies have revealed that conventional glass-ionomer cements may release fluoride into an aqueous environment. However, the sensitivity of cultured human pulp cells to fluoride has not been adequate studied. The objective of this study was to examine the effects of fluoride on human pulp cells *in vitro*. H33258 fluorescence, cell proliferation, protein synthesis and mitochondrial activity assay were used to investigate the pathobiological effects of fluoride on cultured human pulp cells. Fluoride showed cytotoxic effects on human pulp cells during a 24-hr culture period in a dose- and time-dependent manner ($p<0.05$). Elevating the fluoride concentration to 20 ppm almost completely inhibited cell proliferation during 5 day culture period. Fluoride inhibited protein synthesis at 1 mM and higher concentration in a dose-dependent manner ($p<0.05$). In addition, at concentrations of 2 mM through 8 mM, fluoride inhibited 20 % through 44 % of functional mitochondrial activities ($p<0.05$). From the present study, fluoride was found to be a cytotoxic agent to cultured human pulp cells. The cytotoxic effects of fluoride on human pulp cells depended on the exposure dose, frequency, and duration.

P-7

SALIVARY CONCENTRATIONS OF CHLORIDE AND THIOCYANATE AFTER THE CHEWING OF MESWAK. R.A. JALIL*, K. SUSHIL and L.A. SITI. (Faculty of Dentistry, University of Malaya, Kuala Lumpur, MALAYSIA).

The most widely used chewing stick is the meswak which is obtained from the plant, *Salvadora persica* that mainly grows in the Middle East. Meswak is believed to contain substances of value for the prevention of caries and periodontal disease. The objective of this study was to determine the effect chewing of two differently sized commercially available meswak may have on levels of chloride and thiocyanate in whole saliva as opposed to the chewing of an inert material i.e. cotton roll. Twenty subjects participated in this study. They were distributed into two groups (A and B). Subjects in both groups A and B first chewed on meswak (5mm and 10mm diameter respectively) followed by the chewing of an equivalent sized piece of cotton roll (sized #1 and #2 respectively). The titration method was employed for the analysis of chloride whilst thiocyanate levels were determined using spectrophotometer. Higher levels of chloride were registered after meswak chewing compared to cotton roll in both groups A (33.64 mM ± 5.78, 11.48 mM ± 2.07) and B (22.02 mM ± 6.23, 10.42 mM ± 1.02) at $p<0.001$. Although higher levels of thiocyanate were seen in both groups A (0.51 mM ± 0.16, 0.47 mM ± 0.14) and B (0.50 mM ± 0.22, 0.39 mM ± 0.20) after the chewing of meswak compared to cotton roll, the increase was only statistically significant in group B at $p<0.001$. These findings suggest that plants used as chewing sticks may have the potential of releasing substances into saliva that could influence the state of oral health.