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Dentifrice Effects on Plaque Regrowth: Digital Plaque Image Analysis

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In vivo plaque regrowth protocols provide a means for comparing the antiplaque benefits of topical agents (Addy *et al., J Clin Perio* 16: 380-384,1989). In the present study, an *in-vivo* plaque removal/regrowth model was used to evaluate the anti-plaque effects of three dentifrices in a randomized cross-over design. Dentifrices evaluated were: Crest® Gum Care (CGC - 0.454 % SnF_2), Mentadent® Gum Care (MGC - 2.0 % Zinc Citrate/0.76 % SMFP) and Crest® regular (CR - 0.243 % NaF). Nine subjects refrained from oral hygiene for 12 hours prior to presenting to the clinic for baseline plaque measurement via Digital Plaque Image Analysis (DPIA - Sagel et al., this meeting). Next, subjects brushed their entire dentition with 1.5 g. of test product for 1 min. followed by DPIA plaque measurement. Over the next 24 hours, subjects brushed lingual surfaces of their dentition twice more using 1.5 gm of test product. Each brushing included 30 seconds of brushing followed by 30 seconds of swishing the developed slurry to the facial surfaces. Morning plaque levels on day 2 were re-measured via DPIA. Washout period involved 48 hr of placebo (CR) dentifrice use. There were no significant differences between products in terms of plaque removal (initial brushing) ($p = 0.753$). In terms of plaque regrowth, CGC produced a significant 36.8 % reduction in plaque level following 24 hour product use, while MGC (-7.7 %) and CR (-12.9 %) produced no reduction in plaque levels relative to baseline (ANOVA $p=0.0027$). **SnF_2 dentifrice was effective in preventing plaque regrowth between toothbrushings as well as in plaque removal during toothbrushing. The antimicrobial actions of SnF_2 dentifrice were superior under these test conditions to those provided by ZnCit/BS/Peroxide and NaF.**

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Bacterial DNA Changes during Plaque Glycolysis and Regrowth

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Biomass adjustment of bacterial suspensions by turbidity is a key element of PGRM screening (White, *J Clin Dent* 6: 9-70, 1995). Variations in microbial aggregation and the presence of insoluble matrix (glucan) could affect the accuracy of turbidity estimates of plaque bacterial content, particularly following incubation in PGRM tests. This study reports on the precision of plaque bio mass adjustment and plaque regrowth measurement in PGRM made by turbidity. Plaques collected by Texwipe swabbing of PGRM panelists were suspended in buffer solutions using a vortex mixer. Glycolysis or regrowth samples in Eppendorf tubes were dispersed prior to turbidity assessments using a Kontes Pellet Pestle. Initial, post glycolysis or post regrowth media were analyzed for bacterial DNA content using a diphenylamine colorimetric assay following bacteria lysis and extraction (Zameck and Tinanoff, *Arch Oral Biol.*, 32: 807, 1987). A cross-over method calibration study confirmed that PGRM consituents (sucrose, TSB, dextrose, protein, glucan [dextran]) do not interfere with DNA measures which exhibited an RSD of 2.63 %. The bacterial density of PGRM sampled dental plaque measured by DNA assessments is 4.9×10^9 bacteria/mg plaque (dry). A linear relationship was observed between both optical density of plaque dispersions/collected plaque mass ($r^2 = 0.994$) and bacterial DNA for both initial and incubated samples. The minimum detection limit of plaque was determined to be 0.209 mg (dry) which corresponded to an OD (1 ml cuvette) of 0.077 AU (@ 600 nm). **These results confirm that OD adequately acts as surrogate to measure plaque biomass in PGRM and establishes the detection limits available for analysis of dispersed samples for total DNA.**