Effects of Dentifrice Treatments *In Vitro* on *Ex Vivo* Plaque Metabolism

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ABSTRACT

This investigation presents the extension of the previously reported Plaque Glycolysis and Regrowth Model (PGRM) to include more detailed examination of plaque glycolysis kinetics [White et al., *J Clin Dent* 6: 59-70, 1995]. Subjects (no overnight hygiene) sampled a.m. maxillary plaque with a sterilized foam swab and brushed the sampled teeth with 1.5 grams of one of three dentifrices: Crest® Regular (CR), Crest® Gum Care (CGC) or Mentadent® (M). Toothbrushing involved 30 seconds brushing, 30 seconds of swishing of developed slurry followed by a 10 ml water rinse. Following brushing, subjects refrained from eating/drinking and sampled mandibular plaque with a second swab after 45 minutes. Sampled plaques were dispersed in 0.3% TSB and normalized to OD (600 nm) of 0.05 (AU) in 25 ml. The pH of dispersed plaques was adjusted to 7.00 and sucrose added (2%). Glycolysis was measured at 37°C by the potentiometrically controlled pH stat (Brinkman) addition of 0.015 N NaOH. Untreated plaques immediately produced acid at a rate following the equation $[H^+]^{1/2} = kt + c$. The acidogenicity of dental plaques, as defined by the former equation, was relatively constant within subjects (p=0.767) and demonstrated greater variation between subjects (p=0.058). In contrast to untreated plaque, *in vivo* treated plaques typically exhibited a lag period of variable duration before the start of acid production. **Glycolysis for treated plaques (after the initial lag period) were similar to the untreated control plaque--suggesting that treatment effect of the topicals is related to a reversible enzyme inhibition. In terms of toothpaste effects, glycolysis lag times (n=4) measured 88>43>19 minutes for CGC:CR:M (p = 0.097 ANOVA). The pH stat evaluation of ex vivo glycolysis of dental plaque treated in vivo with topical agents may provide unique insights into the mechanism of action and relative activity of antimicrobial formulations.**

INTRODUCTION

The PGRM technique [White et. al., *J Clin Dent* 6: 59-70, 1995] is a useful tool for predicting the *in vivo* antimicrobial effects of plaque agents. The technique allows for rapid *in vivo* screening of products while including important elements of product usage such as absorbtion, diffusion and clearance of the agents. Overnight fasted plaque is sampled with a sterilized foam swab from the subjects maxillary dentition. This plaque serves as the control plaque. The freshly sampled teeth are brushed with a test product and the developed slurry swished throughout the mouth. At subsequent time points, additional plaque samples are obtained by swabbing the remaining treated plaque. All plaque samples are dispersed into 0.3% Tryptic Soy Broth / 2% sucrose and incubated at 37°C. Glycolytic activity of the treated and untreated plaque are measured via pH of the incubation media.

OBJECTIVE

The purpose of this study was to develop a constant pH incubation technique for the PGRM. It is well known that the growth and metabolic activity of plaque bacteria are a function of pH. As bacteria metabolize the sucrose and produce acid, the system becomes self-limiting. Eventually untreated plaque will stop producing acid once the pH is low enough. As a result, agents with a long lasting antimicrobial effect may appear to perform as a placebo product.

MATERIALS AND METHODS

Test Method

1. Subjects refrain from brushing for 12 hours before morning plaque samples are taken. In addition to refraining from oral hygiene, subjects also refrain from eating and drinking the morning of the study until all of the plaque samples have been collected.
2. Using a sterilized Texwipe foam swab, subjects self swab the entire upper dentition (lingual and facial surfaces) and place the swab in a 15 ml sterile centrifuge tube.
3. The subject brushes the sampled dentition with 1.5 grams of the test dentifrice for 30 seconds. After brushing, the subject swishes the developed slurry for 30 seconds. Finally, 10ml of DI water is added to the slurry, swished for an additional 10 seconds and expectorated. No post treatment rinsing was performed.
4. The subject waits 45 minutes while continuing to refrain from oral hygiene, eating and drinking. After 45 minutes, the subject self swabs the lower dentition with a new Texwipe foam swab. As before, the swab is stored in a sterile 15 ml centrifuge tube. At this point, the subject returns to normal dietary and hygiene habits.
5. Approximately 3 ml of 0.3% Tryptic Soy Broth solution is added to each plaque sample and vortexed for 30 seconds. The resultant solution is diluted with additional 0.3% TSB solution to OD (600 nm.) = 0.05 and a final volume of 25ml.
6. The resultant plaque dispersions are added to temperature controlled glass reaction vessels (37°C, 100 ml vessels) and the pH adjusted to 7.00 with dilute HCl.
7. 2% sucrose (0.5 grams) is added to the plaque suspensions and pH maintained at 7.00 through addition of 0.015M NaOH via a Brinkman 719 titrino titrator. The amount of NaOH added to the plaque suspensions is recorded as a function of time with a computer.

Study Design

Three commercial dentifrices were evaluated in a 4 subject crossover design as outlined in the table below:

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	Tuesday	Wednesday	Thursday	Frday
Panelist	***1***	***2***	***3***	***4***
Week 1	CGC	CR	Ment	CGC
Week 2	CR	Ment	CGC	CR
Week 3	Ment	CGC	CR	Ment

CGC = Crest Gum Care
CR = Crest Regular
Ment = Mentadent

RESULTS

Glycolysis profiles for an untreated plaque sample and one treated with Crest® Gum Care are shown in Figure 1. After the initial lag phase, the square root of the acid produced is linear with time as shown in Figure 2. The glycolysis data are well described by the equation $[H^+]^{1/2} = kt + c$.

Figure 1

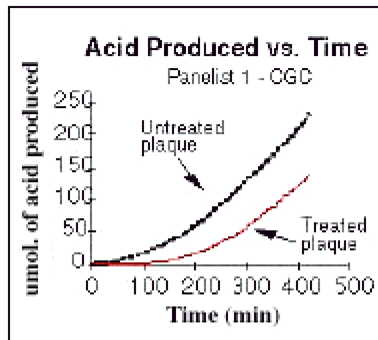
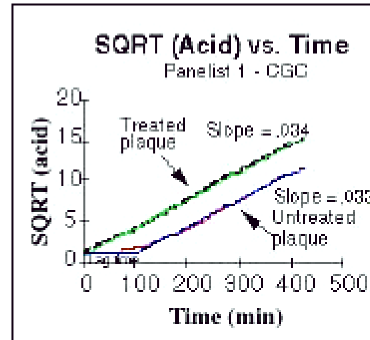


Figure 2



Glycolysis Lag

Crest® Gum Care exhibited a significantly longer glycolysis lag time than Mentadent® (ANOVA, two tail $p=0.040$) and better than Crest® Regular ($p=0.138$). Crest® Regular and Mentadent® were not significantly different ($p=0.400$). The glycolysis lag times are summarized below.

Table 1: Glycolysis Lag Times (Minutes)

Treatment	EC	PS	MS	AG	Avg.
Crest® Gum Care	104	161	50	36	**87.6**
Crest® Regular	64	43	64	0	**42.7**
Mentadent®	25	0	51	0	**19.0**
Average	**64**	**68**	**55**	**11**	**49.8**

Glycolysis Rate (k)

Treated and untreated plaque did not exhibit different glycolysis rates ($p=0.767$). Each subjects' plaque exhibited a unique and constant glycolysis rate across all treatments for the control plaque and treated plaque.

CONCLUSION

-The static pH incubation technique has been successfully applied to the PGRM technique. Acid production is monitored by 0.015M NaOH consumption.

-This extension of the PGRM technique allows glycolysis lag to be observed separately from changes in glycolysis rate. The glycolysis inhibition of dental plaque by Crest® Gum Care appears as an induced lag time rather than a change in glycolysis rate suggesting a reversible enzyme inhibition. None of the three treatments effect glycolysis rate once acid production is initiated.

-Baking Soda/Peroxide appears to be ineffective against glycolytic plaque inhibition.

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