

ABSTRACT

PGRM, a rapid in vivo screening method for antimicrobial effects on plaque (*J Clin Dent* 6: 59-70, 1995), relies upon similar acidogenic and regrowth activity for biomass normalized samples obtained from dentition quadrants. This study reports on microbiological changes occurring during PGRM plaque collection/incubation. Bacterial populations were cultured from PGRM plaque samples (7-10 subjects) obtained from: a) separate dentition quadrants; b) turbidity adjusted TSB growth/glycolysis media; c) post-PGRM growth/glycolysis media. Results demonstrated: 1) similar bacterial populations in samples collected from different dentition quadrants ~ log cfu max. left quadrant: whole mouth = aerobes 7.07(±0.41):7.02(±0.35); anaerobes 7.45(±0.41):7.34(±0.37); total Strep. 7.23(±0.35):7.14(±0.38); Veillonella spp. 5.87(±0.50):5.95(±0.52); 2) no change in bacterial proportions on transfer to PGRM media. Aerobes, anaerobes, Streptococcus spp. and Actinomyces spp. exhibited modest non-significant differences (range from log cfu -0.20 to +0.1) following glycolysis incubation, while remaining Veillonella spp. (-0.46 log) and Fusobacterium spp. (-0.35 log) showed a more substantial change. During regrowth, aerobe, anaerobe and Streptococcus populations increased (~> 1.20 log cfu) while Veillonella (-1.01), Fusobacterium (-0.46) and Actinomyces (-1.57) decreased. These results confirm that different quadrants of the dentition produce similar microbial populations on PGRM sampling; microbial changes in regrowth and glycolysis reflect some preferential selection for aerobic and Streptococcus species as anticipated. Selection of specific media may permit PGRM examination of antimicrobial effects on specific bacterial populations.

INTRODUCTION

The plaque glycolysis and regrowth model (PGRM) allows for rapid in vivo screening of oral antimicrobial formulations. PGRM combines in vivo treatment of overnight accumulated plaque with in vitro metabolic and regrowth analysis of post-treatment plaque samples. The primary advantage of PGRM over existing models is that variations in these measures, typically associated with disparity in plaque biomass, are controlled through normalization of plaque samples collected from different quadrants of the dentition.

Equivalent rates of metabolic activity and regrowth are observed when plaque samples, collected from different quadrants, are similarly dispersed and normalized into incubation medium. The incubation periods for both glycolysis (2 hr) and regrowth (4 hr) were established to provide a wide dynamic range for these measures without permitting untreated controls from reaching equilibrium with respect to either acid production or stationary growth.

PGRM also assumes that a relatively uniform distribution of bacterial species is resident in plaque samples collected from each of the individual quadrants and that these samples are fairly consistent among individuals. These studies were performed to dimensionalize the recovery and distribution of several plaque populations commonly associated with overnight plaque samples. Further, the fates of these populations were assessed following completion of the glycolysis and regrowth incubation periods utilized in the PGRM.

PROTOCOL

Analysis of Viable Organisms in Overnight Fasted Plaque Samples

Ten subjects collected overnight fasted plaque from each upper left (UL), lower left (LL), lower right (LR) and upper right (UR) quadrant using polyester swab applicators. Swabs were dispersed into 2.0 ml of 0.15% trypticase soy broth (TSB). A 0.1 ml aliquot of each sample was diluted into 9.9 ml of 3% TSB. From this dilution, 40 µl was spiral plated on Mitis-Salivarius, Veillonella, and Fusobacterium agars as well as onto 2 trypticase soy agar (TSA) plates. Thirty-six µl was spiral plated onto CFAT agar. The original 1.9 ml sample remaining from each subjects' UL quadrant was normalized to OD₆₀₀ = 0.2 with 0.15% TSB. From each normalized sample, 0.1 ml was diluted and plated as described above.

Analysis of Viable Bacteria Following Glycolysis and Regrowth Incubations

Plaque samples from a single random quadrant were collected over a 4 day sampling period from 7 subjects in a complete crossover design. A portion of each sample was plated before and after normalization to OD₆₀₀ = 0.2 as described above. After normalization, a 1.0 ml aliquot was removed for glycolysis assay. Fifty µl of 40% sucrose was added to the sample and incubated for 2 hr at 37°C with constant, gentle mixing. After incubation, 0.1 ml of each sample was diluted and plated as described above. To evaluate regrowth, a 300 µl aliquot of the normalized plaque was added to a tube containing 0.5 ml of 6% TSB, 100 µl of sterile water, and 50 µl of 40% sucrose. Samples were incubated for 4 hr at 37°C with constant, gentle mixing. After incubation, 0.1 ml of each sample was diluted and plated as described above. All plates were incubated as shown in Table 1. Total aerobes, total facultative anaerobes and total Streptococci colonies were counted using a laser scanner. Veillonella, Fusobacteria and Actinomyces colonies were hand counted.

Table 1. Incubation Conditions for Specific Plaque Bacteria Populations.

Plaque Bacteria	Agar Medium	Condition	Time (hr)
Total Aerobes	Trypticase Soy	aerobic	48
Total Anaerobes	Trypticase Soy	anaerobic	48
Total Streptococci	Mitis-Salivarius	anaerobic/ aerobic	24 (ea.)
Veillonella spp	Veillonella	anaerobic	72
Fusobacterium spp	Fusobacterium	anaerobic	72
Actinomyces spp	CFAT	anaerobic	≥120

Figure 1. Recovery of plaque populations from individual dentition quadrants.

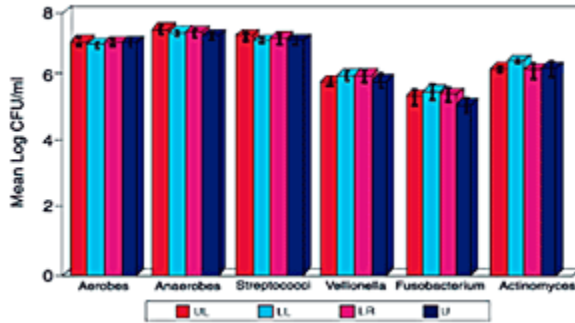


Figure 2. Recovery of various plaque organisms from the UL quadrant relative to the whole mouth.

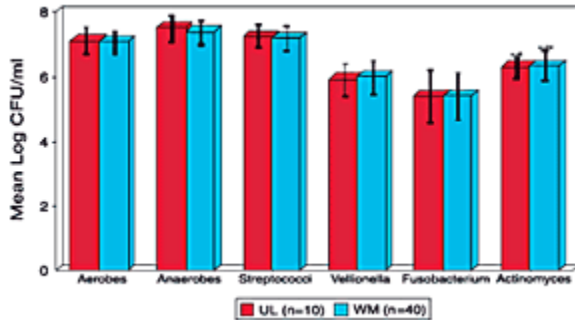
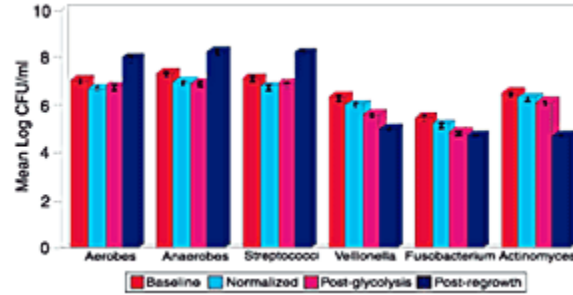


Figure 3. Changes in plaque populations following PGRM normalization, glycolysis and regrowth.



CONCLUSION

-No significant differences were observed in microbial composition or quantity among the 4 distinct quadrants sampled. When compared to the whole mouth, an individual quadrant exhibited no significant differences among the plaque populations screened. Also, normalization of plaque samples did not adversely impact the distribution of plaque organisms recovered from individual quadrants. Thus individual quadrants are representative of the whole dentition and can be measured independently enabling rapid *in vivo* screening of antimicrobial agents.

-Significant decreases were observed among the *Veillonella* and *Fusobacteria* populations after the 2 hr glycolysis incubation period. These results were expected since these organisms tend to be more fastidious in their growth requirements and require a more anaerobic growth environment. No significant changes were observed among the other plaque populations evaluated.

-As expected, significant increases were observed among the total aerobe, facultative anaerobe and *Streptococcus* populations following 4 hr plaque regrowth. Significant decreases were observed among the more fastidious organisms including *Veillonella*, *Fusobacteria* and *Actinomyces* populations