Isothermal Microcalorimetry of Growth of an Oral Streptococcus Species and its Adhesion to Glass Beads

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INTRODUCTION: Bacterial adhesion is the first step in the development of the oral biofilm, called dental plaque. Microorganisms adhere to the saliva-coated human tooth surfaces or dental materials within minutes after tooth cleaning. Plaque is the cause of dental caries, periodontal diseases and peri-implantitis. The range of microorganisms their physiological status and interactions in plaque formation are complex. Previously-published investigations of dental plaque, including bacterial adhesion, employ various in vivo and in vitro models and use microscopic methods to assess surface interactions [1]. Microcalorimetry offers another direct approach, potentially allowing one to measure the energetics of the adhesion process.

METHODS: *Streptococcus sanguinis*, one of the first colonizers, was used as the model organism. TAM III™ thermostats, equipped with microcalorimeters (Thermometric AB, Järfälla, Sweden) were used for isothermal microcalorimetric (IMC) measurements of heat production (µJ/sec=µW) of bacterial growth in Schaedler broth as a function of time expressed by power-time (p-t) curves. To detect heat flow during adhesion of bacteria to glass beads, replication and nearly all metabolic activity were minimized by using stationary *S. sanguinis* suspended in a liquid with either, no nutrition source, PBS, or a very limited one, human saliva.

RESULTS: Replication of an initial concentration of $5x10^6$ *S. sanguinis* cells/ml in Schaedler broth produced a characteristic p-t curve with a maximum of 500 µW when reaching 10^9 cells/ml. A lower concentration of the start inoculum ($5x10^5$ cells/ml) led to almost identical p-t curve demonstrating the high reproducibility of the IMC method. In contrast, 10^9 cells/ml of non-replicating *S. sanguinis* cells, suspended in PBS produced only ~30 µW maximum. But the amount of heat increased with available glass surface area (Table1), indicating that a portion of the heat of adhesion was measured. Stationary *S. sanguinis* cells, suspended in human saliva to mimic conditions of the mouth, resulted in slightly higher energy release. These differences could be related to interactions of proteins or other large molecules in the saliva with the bacteria or residual metabolism rather than to bacteria alone.

Table1. Mean cumulative heat production (µJ) during 1 h adhesion of S. sanguinis (n=10)

Liquid	Glass ampoule	Ampoule +0.3g glass	Ampoule +3.0g glass
PBS	74,800	79,500	91,000
saliva	84,400	94,600	107,000

DISCUSSION & CONCLUSIONS: This study shows that microcalorimetric evaluation of initial bacterial adhesion is indeed possible and may become a rapid and reproducible screening method to study adhesion of different bacteria to different dental materials or modified surfaces. It could be useful to identify bacterial surface molecules involved in adhesion by comparing suitable bacterial mutant strains. It could also be developed into a convenient screening method to compare different dental materials or materials subjected to different surface treatments with the aim of reducing adhesion. Further, it could provide valuable information on interfering substances to be applied in oral prophylaxis.
Detailed results will be published [2].

REFERENCES: [1]I. Hauser-Gerspach, E.M. Kulik, R. Weiger, et al. (2007) *Dent Mater J* 26 (3). [2]I. Hauser-Gerspach, P. Scandiucci de Freitas, AU. Daniels, et al. (2007) *J Biomed Mater Res B Appl Biomater* submitted.

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