Preliminary Investigation of Potential Anti-Microbial Agents Capable of Inhibiting the Growth of Oral Normal Flora

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Introduction

Approximately 500,000 dental implant procedures are performed every year, and this number is growing exponentially [1]. Data have shown that these operations have a failure rate of around 5% [2], where a leading cause of failure is attributable to infection-related complications [1]. It is anticipated that incorporating an anti-microbial agent into a dental implant could combat this issue.

Hypothesis

Chemical agents can have an impact on the metabolism of cells. Therefore, it is hypothesized that exposing oral normal flora to various chemical agents will alter their metabolic activity.

Project Overview

The focus of this experiment was to conduct a preliminary screening procedure for identifying possible chemical agents that exhibit anti-microbial action against oral normal flora. This line of experimentation was facilitated by the utilization of Biolog® 96-well phenotypic microarray (PM) plates. The various sulfur and phosphorous sources and nutrient supplements in the PM plates were tested for their effect on the metabolic activity of oral normal flora.

Methods

Two sets of PM plates were used in this experiment: PM4A and PM5. Four replicates were tested with each set of plates. The PM4A plates contained phosphorous and sulfur sources, and the PM5 plates contained nutrient supplements. In order to measure metabolic activity, a colorless, oxidized redox dye was added to each well of the PM plates. This dye is reduced by active cells, producing a soluble, purple-colored formazan product. The intensity of the purple color is directly proportional to the level of metabolic activity in each well, which was used as a measure of cell growth. The absorbance for each well of the PM plates was measured before and after incubation using a Synergy H1 plate reader (Biotek®) at 600 nm. The difference between these two measurements indicated the effect that each chemical agent had on metabolic activity. For each set of PM plates, an average change in absorbance was calculated for each well. These values were compared to the average change in absorbance for the negative control. If the average change in absorbance measured for a well was significantly less than the negative control, it was concluded that the chemical agent in that well exhibited anti-microbial activity against oral normal flora.

Variables

Controlled variable	Independent variable	Dependent variable
Concentration of bacterial cell suspension	Chemical agent contained within each well of the phenotypic microarray	Metabolic activity of active cells

Procedure

Step 1

Perform oral swab on healthy individual, streak onto blood agar media, & incubate at 37°C for 48 hours.

Step 2

Transfer bacteria from blood agar plate to a conical tube containing Luria-Bertani media.

Step 3

Prepare bacterial cell suspension to an optical density of approximately 0.1.

Step 4

Add redox dye to cell suspension.

Step 5

Inoculate PM plate.

Step 6

Measure absorbance before and after incubating at 37°C for 48 hours.

Results

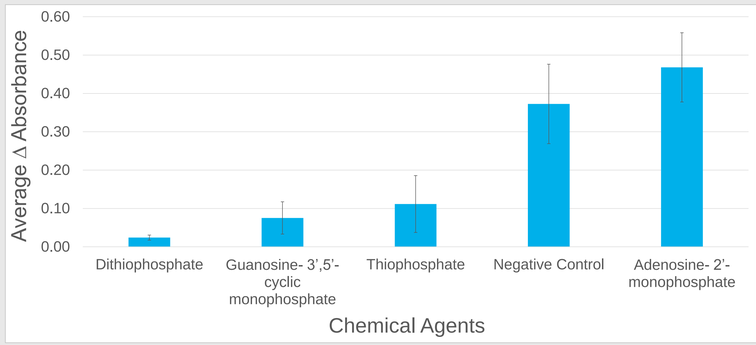

Figure 1. Average Δ absorbance of selected chemical agents in PM4A (phosphorous and sulfur sources). Data presented as average Δ absorbance ± SEM (standard error of the mean).

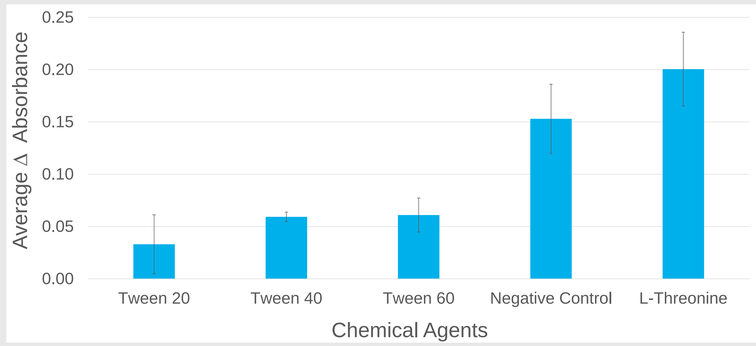

Figure 2. Average Δ absorbance of selected chemical agents in PM5 (nutrient supplements). Data presented as average Δ absorbance ± SEM.

Summary

PM4A Tests

Bacteria grown in the presence of three chemicals (dithiophosphate, guanosine-3',5'-cyclic monophosphate, and thiophoshate) showed a significantly lower level of metabolic activity than the negative control.

Bacteria grown in the presence of all other chemicals on the plate showed similar metabolic activities as adenosine-2'-monophosphate (Figure 1).

PM5 Tests

Bacteria grown in the presence of three chemicals (tween 20, tween 40, and tween 60) showed a significantly lower level of metabolic activity than the negative control.

Bacteria grown in the presence of all other chemicals on the plate showed similar metabolic activities as L-threonine (Figure 2).

Figure 3. Phenotypic microarray prior to incubation.

Figure 4. Phenotypic microarray after incubation.

Conclusion

Results indicate that dithiophosphate, guanosine-3',5'-cyclic monophosphate, thiophoshate, tween 20, tween 40, and tween 60 exhibited anti-microbial activity against oral normal flora. Further testing of these chemical agents is needed to validate this conclusion.

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References

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