INHIBITORY EFFECT OF SOLID SEMICONDUCTOR TITANIUM DIOXIDE ON ACID PRODUCTION BY CARIOGENIC BACTERIA

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ABSTRACT

The inhibitory effect of solid semiconductor titanium dioxide (TiO_2), in the form of rods or chips, on the cariogenic strains of Streptococcus mutans, Actinomyces viscosus, and Actinomyces naeslundii was studied by monitoring total and lactic acid production in a "pH-stat" system. When the TiO_2 rods or chips were used separately in the reaction mixtures, there was no inhibitory effect on both total and lactic acid production by all the strains tested. In contrast, when the TiO_2 rods and chips were used together, both total and lactic acid production were markedly inhibited. Therefore, a combination of TiO_2 rods and chips may have a significant inhibitory effect on acid production when the reaction mixtures are irradiated with visible light.

1. INTRODUCTION

A semiconductor, TiO_2 in the powdered form, has been known to decompose organic compounds, including lactic acid, when it is irradiated with visible light[1,2,3]. The powdered TiO_2 has also a bactericidal effect against some bacterial species[4]. Recently, Morioka et al (1988) have demonstrated that powdered TiO_2 inhibits the viability of S. mutans AHT, when the incubation mixtures are irradiated with light[5]. However, no similar experiments using solid TiO_2 have been carried out. Therefore, the present study was undertaken to examine the effect of solid TiO_2 on the cariogenic bacteria by monitoring production of total and lactic acid in a "pH-stat" system (Radiometer, Copenhgen).

2. MATERIALS AND METHODS

2.1 Bacterial strains and bacteriological procedures

Two strains each of S. mutans (JC-2, PS-14), A. viscosus (251, 262), and A. naeslundii (151, 226) were studied. Each strain was grown anaerobically (10% CO_2, 10% H_2, 80% N_2) in Trypticase soy broth (BBL) at 37°C. The cells were obtained at the mid exponential phase of growth by centrifugation (10,000 g, 10 min at 4°C). The cells were washed with potassium phosphate buffer (0.05 mol/L, pH 7.0), and then resuspended in the same buffer for the preparation of reaction mixtures.

2.2 Preparation of reaction mixtures and incubation procedures

The cells were preincubated anaerobically in a glass jacketed flask at 37°C in a pH-stat system (pH 7.0) to deplete endogenous polysaccharides, as indicated by the cessation of acid production[6,7]. The cells were then washed and resuspended in the buffer and used for the reaction mixtures. In a jacketed glass flask, reaction mixtures were prepared with the following compositions and final concentrations: the cell suspension (4×10^7 cells/mL); glucose at 100 mM (w/v). A total volume of 21 mL of reaction mixtures was then incubated anaerobically in the presence, or absence of either TiO_2 rods or chips, or a combination of both, at 37°C for 30 minutes in a pH-stat system (pH 7.0). The TiO_2 examined was solid Ti metal rod (3 x 53 mm) or chip (0.25 x 1 x 2 mm), coated with a film of TiO_2 (Shiken Co., Osaka, Japan). All reaction mixtures were irradiated with a fluorescent light throughout the incubation period.

2.3 Determination of glucose utilization, and total and lactic acid production

Samples were taken at 2, 4, 6, 8, 10, 15, 20, 25, and 30 minutes, and immediately centrifuged (3,000 g, 10 min at 4°C). Supernatant fractions were analyzed for glucose utilization and lactic acid production, respectively. Glucose concentration was determined by the glucose oxidase method, whereas lactic acid was measured by the colorimetric determination of lactate dehydrogenase. Total acid was measured by monitoring the amount of 0.5 N NaOH required to keep the pH constant during the incubation period. The experiments for each strain were carried out, at least, 3 times. The standard error of the mean is indicated on the graphs. In order to ensure that the numbers of viable cells had remained consistent during the 30-minute incubation period, a 0.5 mL of sample was taken from the reaction mixtures before and after incubation period.

3. RESULTS AND DISCUSSION

3.1 Glucose utilization

In the presence or absence of TiO_2, all the strains examined rapidly utilized glucose during the first ten minutes, and utilized remaining glucose at a progressively decreasing rate thereafter. Glucose was not completely used up by the end of the 30-minute incubation period. The numbers of viable cells remained essentially constant during the incubation period. There were no significant differences in the rates of glucose utilization among S. mutans, A. viscosus, and A. naeslundii species, or between the two strains of each species.

3.2 Total and lactic acid production

When the TiO_2 rods or chips were used separately in the reaction mixtures, there was no inhibitory effect on both total and lactic acid production by the strains examined. In contrast, when the TiO_2 rods and chips were used together, both acid productions were markedly inhibited. As shown in Figures 1-6, there was a continual increase in total and lactic acid production in the absence of TiO_2. In the presence of TiO_2, both acid productions were significantly inhibited. The increased inhibitory effect by a combination of TiO_2 rods and chips may be attributable to the increment in surface area of TiO_2 rods, as well as greater dispersion of TiO_2 chips. The numbers of viable cells during the incubation period remained constant in the presence or absence of TiO2. Furthermore, the rates of glucose utilization by the strains tested were essentially the same regardless of the presence or absence of TiO_2. It is therefore apparent that, under these conditions, irradiated TiO_2 has a selective inhibitory effect on acid production but not glucose uptake by cariogenic bacteria.

4. CONCLUSION

The present study indicates that a combination of TiO_2 rods and chips has a significant inhibitory effect on both total and lactic acid production by the cariogenic bacteria. Acid production, especially lactic acid, by plaque bacteria has been implicated as one of the prime biochemical determinants in the pathogenicity of dental caries. Therefore, further studies as to the exact mechanism(s) involved in the inhibitory effect of solid TiO_2 on acid production should be undertaken. Moreover, the posible clinical applicatons of the solid TiO_2, e.g. toothbrush, are strongly suggested.

5. ACKNOWLEDGEMENTS

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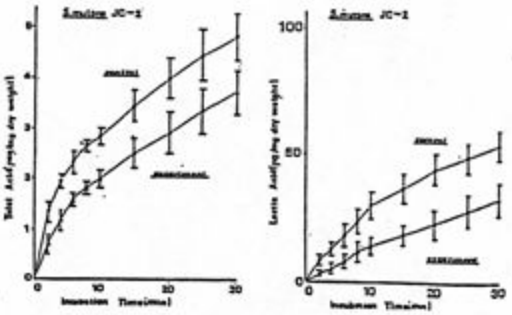

Figure 1. Effect of a combination of TiO_2 rods and chips on total and lactic acid formation by S. mutans JC-2

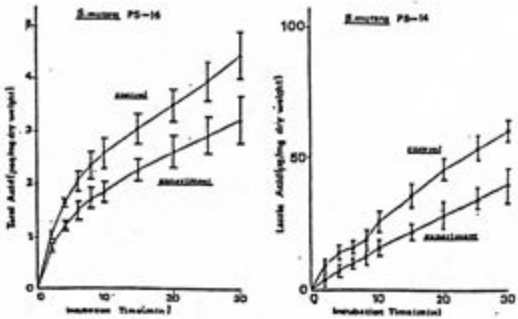

Figure 2. Effect of a combination of TiO_2 rods and chips on total and lactic acid formation by S. mutans PS-14

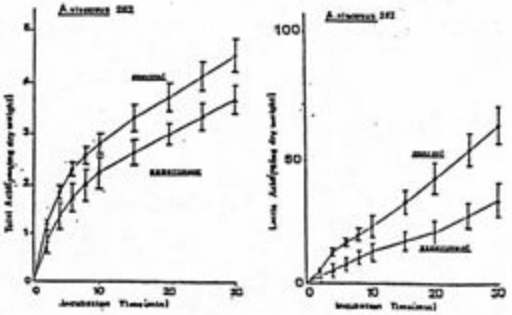

Figure 3. Effect of a combination of TiO_2 rods and chips on total and lactic acid formation by A. viscosus 251

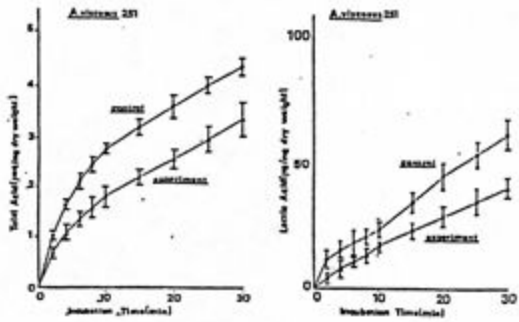

Figure 4. Effect of a combination of TiO_2 rods and chips on total and lactic acid formation by A. viscosus 262

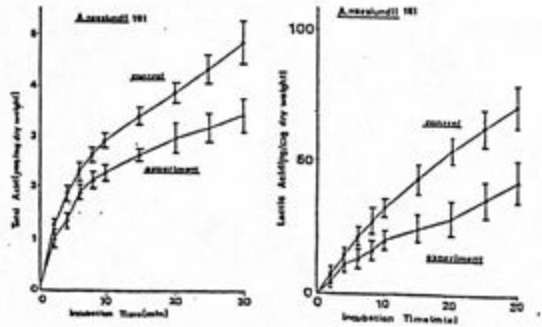

Figure 5. Effect of a combination of TiO_2 rods and chips on total and lactic acid formation by A. naeslundii 151

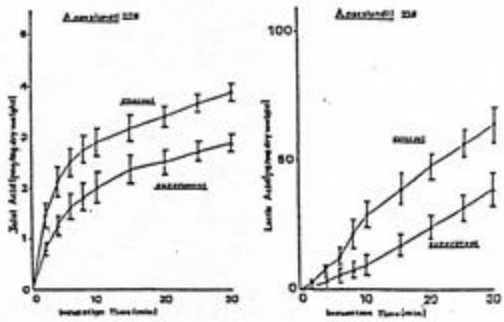

Figure 6. Effect of a combination of TiO_2 rods and chips on total and lactic acid formation by A. naeslundii 226