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Original Article

Effect of Zeta Potentials on Bovine Serum Albumin Adsorption to Hydroxyapatite Surfaces

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Abstract

The aim of the present study was to examine the adsorption of bovine serum albumin (BSA) to hydroxyapatite surfaces by means of zeta potential. The electrophoretic mobility of both hydroxyapatite and BSA were negative, with BSA itself less negative than hydroxyapatite. The zeta potential of the surface of BSA-adsorbed hydroxyapatite was significantly more negative than that of hydroxyapatite alone ($p < 0.0001$). The BSA histogram indicated two negative peaks, and the zeta potential of BSA-adsorbed hydroxyapatite also showed two similar negative peaks. These results suggest that BSA adsorption to hydroxyapatite surfaces is related to electrostatic interaction.

Key words: Zeta potentials—Hydroxyapatite—Bovine serum albumin—Adsorption

Introduction

The forces that affect various behavioral characteristics, including the adsorption and desorption of microorganisms, proteins, and other substances at interfaces can be broadly classified into 2 types: 1) the forces which act between the molecules composing a substance (hydrophobicity); and 2) electrical interactions resulting from surface charges. The hydrophobicity of the surface of a substance can be qualitatively evaluated by measuring contact angles and surface free

energy. Surface potentials, on the other hand, are more difficult to measure. Therefore, surface electrokinetic potentials (zeta potentials) are calculated and quantified on the basis of electrophoretic mobility⁵). Changes in surface charge density can be determined by measuring zeta potentials at before and after adsorption, thereby allowing adsorption capacity to be calculated. This principle has been applied in the development of materials for artificial organs, where it is used to evaluate the adsorption of proteins, enzymes, and other biological macromolecules, study the effects

of washing on protein removal, and investigate the prevention of adsorption by coating³⁾.

We previously studied the mechanism underlying the adsorption of oral bacteria to the surfaces of prosthetic materials. Our results showed that electrostatic interactions have a role in the adsorption of oral bacteria to the surfaces of prosthetic materials such as ceramics and resins. In addition, zeta potentials were demonstrated to be important factors in the adsorption of oral bacteria to prosthetic materials⁸⁾.

The aim of the present study was to examine the adsorption of bovine serum albumin (BSA), which was used to block the adsorption of bacteria to experimental salivary pellicles⁹⁾, to hydroxyapatite surfaces by means of zeta potential.

Materials and Methods

The zeta potential of hydroxyapatite alone, BSA alone, and BSA-adsorbed hydroxyapatite surfaces were determined. The role of electrostatic interactions in the adsorption of BSA to hydroxyapatite surfaces was investigated based on the zeta potentials obtained.

1. Materials

1) Hydroxyapatite plate materials

Sintered hydroxyapatite pellets (Apatite PelletTM, Pentax Co., Tokyo, Japan) measuring 20×40×2 mm (APP-ND033C, Lot No. D03K2DEF) were used.

2) Bovine serum albumin

Bovine serum albumin (Sigma-Aldrich Co., St. Louis, Missouri, USA) was used to examine the adsorption of proteins to hydroxyapatite plate materials. Bovine serum albumin (2.0×10⁻⁵ mol/liter) was dissolved in 10 mM sodium chloride solution to the desired concentration. The pH of a 10 mM sodium chloride solution in equilibrium with the atmospheric pressure of carbon dioxide was 5.6.

2. Methods for producing materials

The surface was finished with waterproof abrasive papers (#180, #320, #600, and #1200).

The materials were then buffed with alumina abrasives (5 μm and 0.05 μm) to achieve a plate-like finish. Ultrasound cleaning was then performed for 15 min. The materials were preserved in a desiccator for 1 week.

3. Measurement of zeta potentials

1) Hydroxyapatite plate materials

An electrophoretic light-scattering spectrophotometer (ELS-800TM, Otsuka Electronics Co., Ltd., Hirakata, Japan) that can measure the surface potentials of plate-like materials was used to measure the zeta potentials of the hydroxyapatite plate materials. Polystyrene latex spheres (particle diameter, 520 nm, Otsuka Electronics Co., Ltd.) coated with hydroxypropyl cellulose (MV=3.0×10⁶, Scientific Polymer Products Inc., Ontario, New York, USA) were used as monitor particles and suspended in 10 mM sodium chloride solution (pH 5.6). This coating was chosen as it would not interact with the materials during measurements.

In solutions with high electrical conductivity, stronger electric fields are associated with higher heat values. Therefore, electrophoresis is difficult in highly concentrated solutions⁹⁾. Given that buffer solutions and pH influence zeta potentials after adsorption of BSA¹⁴⁾, 10 mM sodium chloride solution was used instead of phosphate buffered saline solution, which had been used previously⁸⁾.

2) Bovine serum albumin and monitor particles

A zeta potential analyzer (ZEECOM ZC-2000TM, Microtec Co., Ltd., Chiba, Japan) was used for microscopic electrophoresis to measure the zeta potentials of BSA and monitor particles. The movement of particles during electrophoresis was monitored microscopically and displayed on a monitor. Distances between moving particles underwent image processing on a personal computer, and zeta potentials were automatically calculated¹⁰⁾. To compare measured values among devices with different properties, the zeta potentials of the monitor particles were also measured.

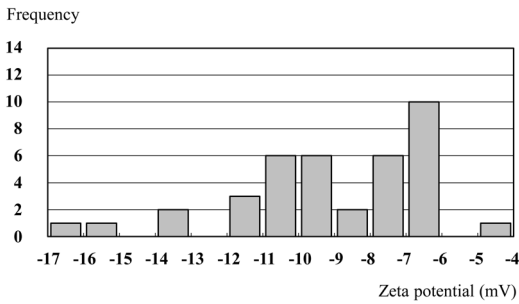


Fig. 1 Zeta potentials of hydroxyapatite

Values indicate zeta potential of hydroxyapatite surfaces from 38 specimens.

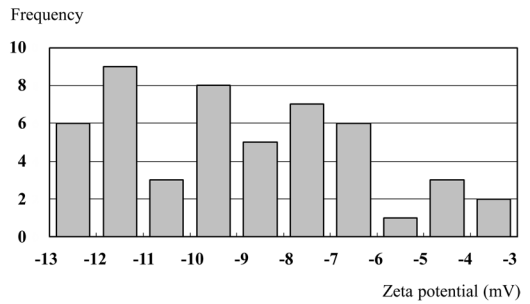


Fig. 3 Zeta potentials of monitor particles

Values indicate zeta potential of monitor particles from 50 specimens.

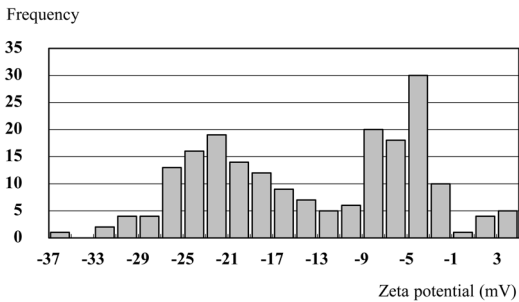


Fig. 2 Zeta potentials of BSA

Values indicate zeta potential of BSA from 200 specimens. Histogram showed 2 peaks.

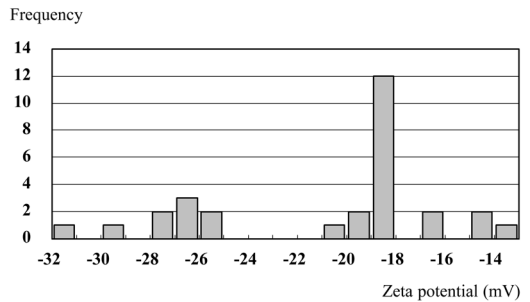


Fig. 4 Zeta potentials of hydroxyapatite surfaces after BSA adsorption

Values indicate zeta potential of hydroxyapatite surfaces after BSA adsorption in 18 specimens. Histogram of results showed 2 peaks, similar to BSA.

4. Measurement of zeta potentials of hydroxyapatite surfaces after BSA adsorption

Bovine serum albumin (2.0×10^{-5} mol/liter) was suspended in 10 mM sodium chloride solution (pH 5.6) and allowed to stand for 30 min. The cell and tube systems were then washed with approximately 50 ml 10 mM sodium chloride solution. Monitor particles in 10 mM sodium chloride solution were then injected into the tubes for measurement of zeta potentials. Zeta potentials before adsorption were compared with the values at after adsorption.

5. Statistical analysis

Experimental data were analyzed with the use of SAS/STAT software, version 9.1, for UNIX (SAS/STAT, SAS Institute, Cary, North Carolina, USA). The Wilcoxon rank-sum test was used to compare zeta potentials.

Results

The mean zeta potential of hydroxyapatite surfaces was -9.0 ± 2.7 mV (range, -16.6 to -4.3 mV, $n=38$, Fig. 1). The mean zeta potential of BSA was -14.5 ± 9.4 mV (range, 3.6 to -36.1 mV, $n=200$, Fig. 2). Histograms of the data showed 2 peaks. The zeta potential of BSA after subtracting the zeta potential of the monitor particles (-8.9 ± 2.5 mV, $n=50$, Fig. 3) was -5.6 mV.

The mean zeta potential of hydroxyapatite surfaces after BSA adsorption was -22.2 ± 5.8 mV (range, -31.1 to -13.9 mV, $n=18$, Fig. 4). The histogram of the results showed 2 peaks, similar to BSA. The zeta potential after BSA adsorption was significantly more negative than the zeta potential of hydroxyapatite surfaces ($p < 0.0001$).

Discussion

Previous studies have used zeta potentials to examine the adsorption of saliva proteins and oral bacteria in detail^{2,12,14}. However, most studies measuring zeta potentials have employed microscopic electrophoresis and crushed dental materials as the adsorbent and synthetic hydroxyapatite powder as the enamel substitute. In contrast, we used solid adsorbents to experimentally examine adsorption phenomena, assuming that surface properties differ between powders and solids and that adsorption of saliva proteins and oral bacteria occurs at solid surfaces as well as liquid interfaces.

To measure the zeta potential of BSA, we selected particles of less than 300 nm in diameter, confirming size by laser analysis. Longworth⁶ estimated that the isoelectric point of BSA was $pH_0 = -4.89$. Although a different solvent was used in the present study, a similar value was obtained.

In a study by Young¹⁵, the zeta potentials of human enamel and hydroxyapatite particles became less negative after adsorption of parotid or whole saliva. In a study by Shimomura¹¹, the zeta potential of the surfaces of hydroxyapatite plates became less negative after protein adsorption. Although the proteins and solvents used in these studies differed from those used in the present study, zeta potentials were reported to reflect protein levels after their adsorption to the surfaces of hydroxyapatite plates, similar to in the present study. However, those studies did not measure the zeta potentials of the proteins used or clarify whether protein adsorption was caused by electrostatic interactions or hydrophobicity.

Bierman¹ reported that when equivalent electrical double layers of the same charge approach each other, electrostatic interactions cause repulsion, thereby increasing zeta potentials. In our study, the zeta potentials of hydroxyapatite surfaces and BSA were all negative, and the zeta potential of BSA was less negative than that of hydroxyapatite. Zeta potentials after BSA adsorption were

significantly more negative than those of hydroxyapatite surfaces. Histograms of the zeta potentials of BSA alone showed 2 peaks (Fig. 2). Similar to the zeta potentials of BSA alone, the histogram of the zeta potentials of apatite surfaces after BSA adsorption also showed 2 peaks (Fig. 4). The results of the present study were consistent with the theoretical characteristics of electrostatic interactions.

Morisaki⁷ found that the cell surface of bacteria isolated from a soil environment is negatively charged. They also found that bacterial strains with slower proliferation rates have smaller absolute surface charge values, higher hydrophobicity, and stronger adhesive capacity. One study attempted to investigate the adhesion of oral bacteria to dental restorative materials, focusing on surface free energy¹⁵.

Further study is needed to qualitatively evaluate the hydrophobicity of substance surfaces by measuring contact angles and surface free energy. We believe that evaluating the adsorption of proteins, enzymes, and other biological macromolecules to the surfaces of prosthetic materials, studying the removal of proteins by washing, and preventing adsorption by coating would contribute to the development of prosthetic materials.

Conclusions

The zeta potentials of hydroxyapatite alone, BSA alone, and the surfaces of BSA-adsorbed hydroxyapatite *in vitro* were measured. The findings revealed the following concerning the role of electrostatic interactions:

1. The zeta potential of BSA was -5.6 mV.
2. The zeta potential of hydroxyapatite surfaces was -9.0 mV, and the value after BSA adsorption was -22.2 mV, which was significantly more negative than the value at before BSA adsorption ($p < 0.0001$).
3. Histograms of the zeta potentials of BSA alone showed 2 peaks. Histograms of the zeta potential of hydroxyapatite surfaces after BSA adsorption also showed 2 peaks, similar to the histograms of BSA alone.
4. These results suggest that electrostatic

interactions have a role in the adsorption of BSA to hydroxyapatite surfaces.

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