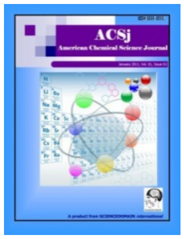

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A Comparative Study of the Adsorptive Characteristics of Mucin to Calcium Hydroxyapatite and Titanium Implants

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ABSTRACT

Aims: The study is aimed to examine the adsorptive characteristics of the main salivary protein, mucin onto Ti surfaces and compare the scientific data with that onto CaHap; towards elucidating the biocompatibility of these two candidates in biomedical applications.
Place and Duration of Study: Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria. Study was carried out between January and April, 2004.
Methodology: To 50 mg, 100 mg, 150 mg and 300 mg of Ti or CaHap powder, 10 mL of (1% or 5%) mucin solution already incubated at 37^{o}C was added at a contact time of 45 minutes in an incubator. After discarding the supernatant, a 10 mL volume of doubly distilled water was added to each residue and rinsed to remove the unadsorbed mucin. The adsorbent particles with the adsorbed mucin of an experimental group was then heated to 60^{o}C for five hours in an oven and then weighed. The samples were then placed in a muffle-furnace at 600^{o}C for 30 minutes to remove the mucin by burning, so as to obtain the weight of adsorbed mucin.
For the experiment on the adsorptive strength, to seven sets of 500 mg Ti or CaHap powder 10 mL of 1% mucin solution was added in an incubator. After centrifugation, the supernatant solution above the titanium particles was removed from the precipitates, and 8

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mL of distilled water incubated at 37^{o}C added; after shaking, the supernatant was again removed by the same method. The same rinsing procedure as described above was repeated 0, 1, 2, 4, 8, 10 and 12 times in different experimental groups. The Ti or CaHap particles were heated to 60^{o}C for 5 hours in an oven and weighed. The adsorptive strength was evaluated by comparing the weight change between the no rinse and multiple rinse samples.
One gramme of Ti or CaHap powder placed in test tubes was suspended in 1.0 mL of solutions containing 0.2 – 1.8 mg/mL mucin solution. Following constant shaking for 24 hours at 37^{o}C, the suspensions were allowed to settle and the supernatants collected to determine the adsorption isotherms. Bradford assay was performed on 0.1 mL supernatant samples to obtain Langmuir adsorption isotherm for the materials.
Results: The amount of mucin adsorbed to CaHap was 1.79 ± 0.65 times higher than that to Ti. At the eighth rinse mucin particles remain adsorbed to CaHap but none on the Ti surfaces. The adsorption isotherms of mucin onto both materials exhibited the Langmuir adsorption isotherm, with a maximum of 0.14 mg mucin adsorbed/ 1.0 g CaHap powder compared to a maximum of 0.11 mg mucin/ 1.0 g Ti powder reported by Lori and Nok, (2004).
Conclusion: Mucin can be completely rinsed from the surfaces of artificial titanium tooth and braces deployed in the mouth, thereby prolonging the lifespan. Adsorption of mucin onto these two biomaterial surfaces exhibited the Langmuir type with similarity, so bio-engineered Ti-Hap composite has excellent biocompatibility. The results of these *in vitro* experiments were consistent with the proposal that ceramics have a higher adsorptive ability than do metals.

Keywords: Calcium hydroxyapatite; titanium; mucin; adsorption; isotherm;

1. INTRODUCTION

The adsorption of proteins on the surface of a biomaterial is a fascinating and complex process that stands as the most important issue in evaluating their biocompatibility (Mura-Galelli et al., 1991). Mucin is a glycoprotein that covers the surface of the buccal cavity and epithelial organs.

Calcium hydroxyapatite $Ca_5(PO_4)_6(OH)_2$, CaHap becomes essentially suitable for implantation since it is chemically close to calcium phosphate, that is the mineral phase of bone (Constantino et al., 1992; Sun et al., 1998; Wang et al., 1994).

Osseointegrated titanium (Ti) implant has become the biomaterial of choice in dental restoration and is in general well suited for bone anchorage applications. Titanium reacts immediately with oxygen on exposure to air, forming a 5-10 nm surface oxide layer. Titanium dioxide has physico-chemical characteristics that differ from metallic titanium, characteristics which are more closely related to ceramics than to metals (Johansson et al., 1990).

Apatite (Hap) coated titanium components which combine the advantages of the mechanical strength of Ti metal and the bioactivity of Hap are developed and reckoned to be one of the most promising groups of implant materials in orthopeadic and dental fields (Ma et al., 2003).

This paper examines the adsorptive characteristics of the main salivary protein, mucin onto Ti surfaces and compares the scientific data with that onto CaHap. A comparative evaluation of the adsorption of proteins onto Ti and CaHap is of great interest in elucidating the biocompatibility of these two candidates in biomedical applications towards the design of engineered Ti-CaHap composite optimised to bind mucin for biocompatibility.

2. MATERIALS AND METHODS

2.1 Materials

Titanium metal powder of particle size 500μm was obtained from BDH chemicals Ltd. (Poole, England). Its purity was verified by atomic absorption spectroscopy (Buck Scientific 200A) and was found to contain about 99.7 ± 0.01% Ti.

CaHap with a particle size ranging from 5-15μm was obtained from sigma-Aldich Co., USA. Mucin, (Nacatai, Tesque Inc., Kyoto, Japan. Batch no. MIP960) was the protein used for the adsorption studies.

2.2 Thermal Stability of Titanium and Calcium Hydroxyapatite

Titanium powder of mass 50 mg each in three crucibles was heated at 600°C for 30 minutes in a muffle- furnace. This was also done for CaHap particles.

2.3 Protein Adsorption Studies

2.3.1 Adsorptive amount of mucin

To each of the 50 mg, 100 mg, 150 mg and 300 mg of Ti or CaHap powder weighed into centrifuge tubes, 10 mL of (1% or 5%) mucin solution already incubated at 37°C was added. The solutions were continuously shaken for 45 minutes in an incubator (1H-150, Gallenkamp Co., England) at 37°C. Centrifugation was carried out and the supernatant removed. A 10 mL volume of doubly distilled water was added to the residue in each of the centrifuge tubes and rinsed. The rinsing was to remove the unadsorbed mucin. The supernatant was removed again after centrifugation. The adsorbent particles with the adsorbed mucin of an experimental group was then heated to 60°C for five hours in an oven and then weighed by using an analytical balance (H15, E. Mettler Co., Switzerland, accuracy 10^{-4} g).

The samples were then placed in a muffle-furnace at 600°C for 30 minutes to remove the mucin by burning. The weights of the samples were again determined using a Mettler balance. The weight of adsorbed mucin was calculated by comparing the weight after drying at 60°C and after heating to 600°C.

2.3.2 Adsorptive strength of mucin

Seven sets of 500 mg Ti or CaHap powder were weighed into centrifuge tubes and 10 mL of 1% mucin solution was added to each (Niwa, 1999). The incubation, centrifugation and rinsing were carried out. After centrifugation, the supernatant solution above the titanium particles was removed from the precipitates, and 8 mL of distilled water incubated at 37°C added; after shaking, the supernatant was again removed by the same method. The same rinsing procedure as described above was repeated 0, 1, 2, 4, 8, 10 and 12 times in different experimental groups. The Ti or CaHap particles were heated to 60^{o}C for 5 hours in an oven and weighed. The samples were then cooled in a dessicator and weighed again. The adsorptive strength was evaluated by comparing the weight change between the no rinse and multiple rinse samples.

2.3.3 Langmuir adsorption isotherm of mucin adsorbed on CaHap and Ti

One gramme of Ti or CaHap powder placed in test tubes was suspended in 1.0 mL of solutions containing 0.2 – 1.8 mg/mL mucin solution. Following constant shaking for 24 hours at 37^{o}C, the suspensions were allowed to settle and the supernatants collected to determine the adsorption isotherms. Bradford assay was performed on 0.1 mL supernatant samples. The amount of adsorbed protein was calculated by subtracting the amount of unadsorbed (free) protein remaining in the supernatant from the amount of protein in the control (mucin not suspended in Ti or CaHap powder sample) and plotted in the form of a Langmuir adsorption isotherm. The maximum amount of adsorbed mucin, the mucin-Ti and the mucin-CaHap association constants were calculated from the slope and the x-intercept respectively of the linear curve.

$$F/_B = 1/_{K_\propto N} + 1/_N . F$$

Where B = bound mucin; F=free mucin; $K_\propto$ = association constant and N = maximum amount of mucin adsorbed

3. RESULTS AND DICUSSION

Data are expressed as the average of triplicate determinations ± the standard error of the mean.

3.1 Thermal Stability

Titanium does not undergo decompositional change at 600^{o}C, so its weight remains unchanged. CaHap on the other hand losses 3.8% of its original weight by being heated at 600^{o}C, this correction factor was put into cognisance in the desorption process.

3.2 Amount of Adsorbed Mucin

The amount of (1%) mucin adsorbed (mg) onto CaHap was 7.30 in the 50 mg group; 14.80 in the 100 mg group; 15.20 in the 150 mg and 19.30 in the 300 mg group. For Ti, the amount of (1%) mucin adsorbed (mg) was 4.20 in the 50 mg group; 6.70 in the 100 mg group; 9.60 in the 150 mg group and 12.70 in the 300 mg group as shown in Figure 1. As presented in Figure 2, the amount of mucin adsorbed (mg) onto CaHap was 16.40 in the 50 mg group; 23.70 in the 100 mg group; 31.20 in the 150 mg group and 44.20 in the 300 mg group by the use of 5% mucin solution. The amount of adsorbed mucin (mg) onto Ti by the use of 5% mucin solution was 12.00 in the 50 mg group; 12.50 in the 100 mg group; 13.10 in the 150 mg group and 25.10 in the 300 mg group.

The results showed that the weight of mucin adsorbed onto CaHap was 1.79 ± 0.65 times higher than that onto Ti powder. Statistical analysis by Duncan's one way ANOVA showed that there was no significant difference in the amount of mucin adsorbed onto CaHap and that onto Ti ($P < 0.05$).

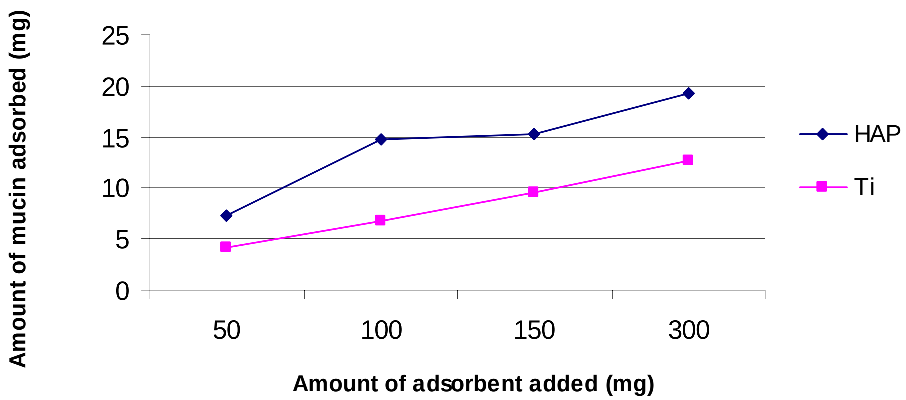

Fig. 1. Adsorption of 1% mucin solution to CaHap and Ti

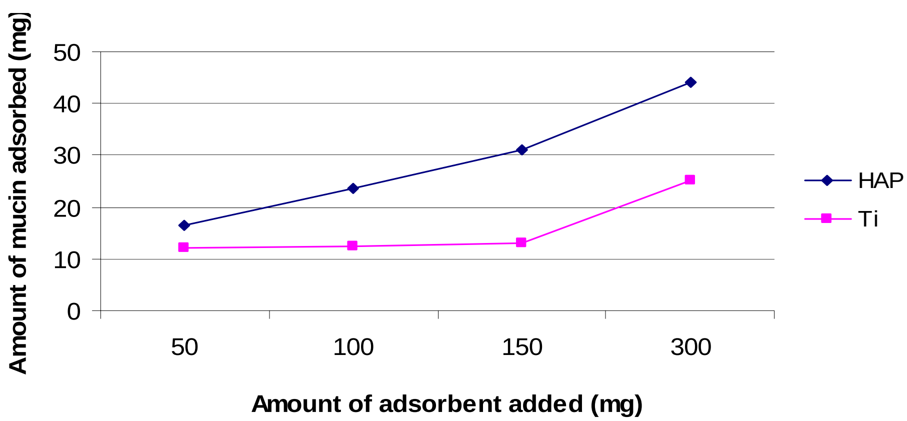

Fig. 2. Adsorption of 5% mucin solution to CaHap and Ti

3.3 Adsorptive Strength to Mucin

The proportion of mucin adsorbed onto CaHap was 29.50% without the rinsing being carried out, while the proportions were 28.20%, 26.70%, 21.90%, 16.20%, 16.20% and 16.10% with 1, 2, 4, 8, 10 and 12 rinses respectively. The proportion of mucin adsorbed onto Ti were 8.40% without rinsing while for 1, 2, 4 and 8 rinses the values were 2.30%, 0.80%, 0.004% and 0.00% respectively as indicated in the Figure 3.

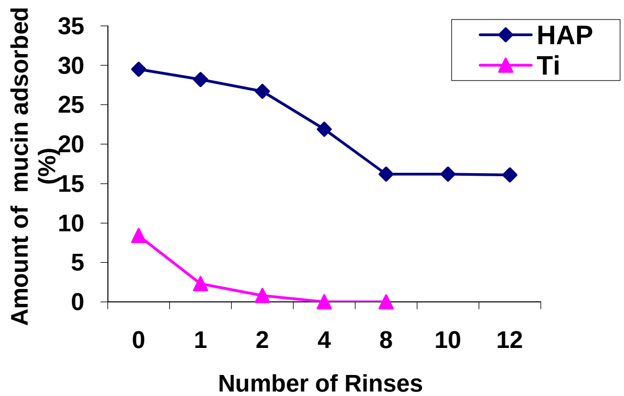

Fig. 3. Adsorptive strength of CaHap and Ti to mucin

3.4 Langmuir Adsorption Isotherm

Increasing the concentration of mucin from 0.2 to 1.8 mg/mL resulted in increased adsorption of mucin onto CaHap. Langmuir adsorption isotherm showed linearity of the adsorption process as reported in Figure 4, with a maximum of 0.14 mg mucin adsorbed/ 1.0 g CaHap powder which was compared with the report of Lori and Nok (2004) given as a maximum of 0.11 mg mucin/ 1.0 g Ti powder in Figure 5 (N = 1/slope). The mucin-CaHap association constant was 0.05 ml/mg while the mucin-Ti association constant was 2.91ml/mg from the study by Lori and Nok (2004) (K = - X intercept).

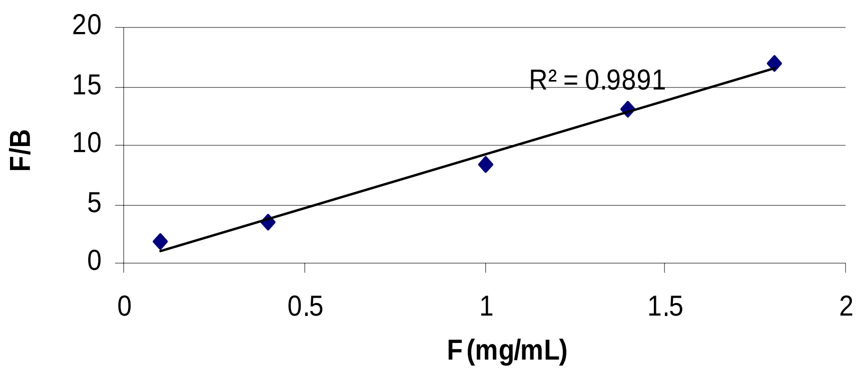

Fig. 4. Langmuir adsorption isotherm of mucin onto CaHap

The study showed the amount of mucin adsorbed onto CaHap as being 1.79 ± 0.65 times higher than that to Ti. The discrepancy can be attributed to the use of metallic titanium. Johanson (1990) demonstrated that titanium oxide surfaces show a possible similarity in the adsorption of protein to that by CaHap . The mechanism of adsorbing to organic substances by apatite is considered to be related to hydrogen bonding and zeta potential change between adsorbent and substrates which are adsorbed (Niwa, 1999). The adsorption kinetics of bovine serum albumin, BSA molecules was reported to strongly adsorb onto CaHap mainly through a specific electrostatic attractive force between negative charges on BSA and localised positive ones on CaHap surfaces (Kazuhiko, 2000). Since mucin is an acidic protein like BSA the mechanism of adsorption follows the same pattern. The mucin binding to titanium surfaces is speculated as being that the surface-exposed carboxyl group may be attracted to the oxide covered surface of titanium by electrostatic interaction.

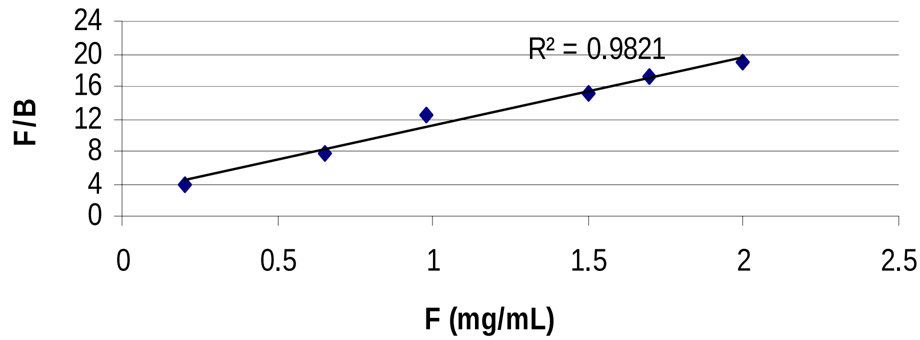

Fig. 5. Langmuir adsorption isotherm of mucin onto titanium

Hydrophilicity of the titanium – linked O^- surface makes it to attract water and could be another suggested factor for its lower adsorptive capacity than does CaHap, which is hydrophobic.

Desorption of the mucin molecules by multiple rinsing is a pointer to the bonding strength existing between mucin molecules and the two adsorbent media. As presented in Figure 3, mucin molecules are still firmly adsorbed onto CaHap surfaces after the eighth rinse. On the other hand, all the mucin adsorbed onto the Ti particles was removed at the eighth rinse. The adsorption of mucin onto Ti is therefore by physical adsorption while that to CaHap is by chemisorption. The binding strength of mucin onto the Ti or CaHap surface is of importance, strongly bound proteins will consistently hide the surface while weakly bound proteins can be more easily replaced by other molecules. This report re-affirms mucin as an adhesive salivary and lymphatic glycoprotein.

4. CONCLUSION

Mucin can be completely rinsed from the surfaces of artificial titanium tooth and braces deployed in the mouth, thereby prolonging the lifespan.

The adsorption isotherms of mucin onto these two biomaterial surfaces exhibited the Langmuir type. A similarity in these adsorption isotherms is of great interest in elucidating the observed excellent biocompatibility of Ti-Hap composite biomaterials. The results of these *in vitro* experiments were consistent with the proposal that ceramics have a higher adsorptive ability than do metals.

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