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Effect of pH, cations and incubation time on the adsorption of globulin onto titanium

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ABSTRACT

In these studies, the effect of pH, cations, and incubation time on the adsorption of globulin onto titanium was studied invitro. Globulin adsorption onto commercially pure titanium power that was pretreated with calcium, magnesium or potassium ions was carried out at pH 3.0 and pH 7.0. The amount of globulin adsorbed was calculated using the value of the regression line obtained from the standard Bovine serum Albumin (BSA) calibration curve. The pretreatment of titanium with calcium and magnesium alone or combined, with increasing pH valves (3.0 - 7.0) resulted in an argumented adsorption of globulin onto titanium. No increase in adsorption was observed following the pretreatment of titanium with potassium. The incubation time was also studied extensively and the result in various research work shows that there is involvement of electrostatic interaction in the adsorption of globulin to titanium.

KEYWORDS

Titanium;
Bovine serum albumin (BSA);
Globulin;
Adsorption;
Cations;
Biuret reagent.

INTRODUCTION

Titanium is widely used by dental professionals to anchor prosthetic appliance, and in recent years osseo-integrated titanium implants have become the method of choose since close contact between bones and implants have been demonstrated various studies[1-4,9]. Hence owning to its high dielectric constant, TiO_2 undergo further modification upon binding surrounding ions. These may include calcium, potassium and hydrogen which may result to the generation of OH radicals in oxide. The oxide layer may adsorb macromolecules from the implant vicinity including tissue component and protein[14]. So when a foreign material is implanted into a host tissue, the first event to occur at tissue-metal interface which dictates bio-compatibility is a non-covalent adsorption process of proteins from the body onto the titanium metal surface[5,6].

In previous studies[3], it was showed that the chemical properties of TiO_2 suggested that Ca^{2+} may be attracted to oxide covered surface by electrostatic interaction with O_2 and hence calcium deposits have been observed in direct context with TiO_2. This is due to the fact that titanium react immediately with oxygen when expose to air to form 5 - 6nm surface oxide layer, and this layer which increasing during prolong exposure to oxygen consist primarily of TiO_2[13,19]. Therefore tissue implant reaction is then a reaction with TiO_2 at implant surface and not with element titanium as such due to the fact that they have physical chemical characteristics related to ceramics than to metal, establishing that titanium bind cations[19,21].

The adsorption of globulin onto titanium is however often a highly dynamic phenomenon and the molecules may change orientation and conformation during or after adsorption. Although studies are on to develop simple models for protein adsorption based on geometrical area covered by protein molecules in different state[18].

MATERIALS AND METHODS

All reagent used were Analar grade. Third-five mesh of commercially pure Titanium grade II power was obtained from bovine blood and commercially pure which was 99% electrophoresis and contains less than 4% NaCl manufactured in U.S.A. Bovine Serum Albumin (BSA) consisting of albumin, flourescin, isothiocyanate conjugated bovine as stable at 2 - 8°C. Biuret reagents and disodium phosphate buffer e.t.c.. 1.0mg/ml of BSA stock was use to prepare the calibration curve. The absorption was taken at 540nm using JENWAYS UV/Visible spectrophotometer. A regression line equation was obtains which was used to calculate the amount of globulin adsorbed.

Effect of cations on the adsorption of 1mg/ml globulin onto titanium was done by suspending 0.5g of titanium powder for 48hrs at 27⁰C in 0.1M of the cations ($CaCl_2$, $MgCl_2$ and KCl) respectively. All samples were then filtered, washed with distilled water and dry at room temperature for 48hrs. The samples were then suspended in 1ml solution containing globulin and shaken at 37⁰C for 2hrs in 2XM10/250 Model Gallenhamp Incubator with a shaker, after which 4ml of biurets reagents was added to each sample. The resulting mixture was mixed thoroughly by inversion and allowed to incubate at room temperature for 30mins.

Also, effect of incubation time on the adsorption of 1mg/ml of globulin onto titanium and treated-titanium was done by suspending 0.05g of titanium powder at room temperature in distilled water and 0.1M $CaCl_2$ respectively. All samples were then filtered, washed with distilled water and dry at room temperature for 48hrs. The samples were then suspended in 1ml solution containing globulin and shaken at 37⁰C for 2hrs in 2XM10/250 Model Gallenhamp Incubator with a shaker, after which 4ml of biurets reagents was added to each sample. The resulting mixture was mixed thoroughly by inversion and allowed to incubate at 37⁰C for 10, 20, 30, 40 and 50hrs respectively.

RESULTS

The effect of cations on adsorption of globulin onto titanium is presented in Figure 1. The results revealed high effect of Ca followed by Mg then K. The effect of Ca-Ti and Mg-Ti were similar compared to K-Ti. The effect of cations-Ti on globulin showed the following patterns Ca-Ti > Mg-Ti >> K-Ti.

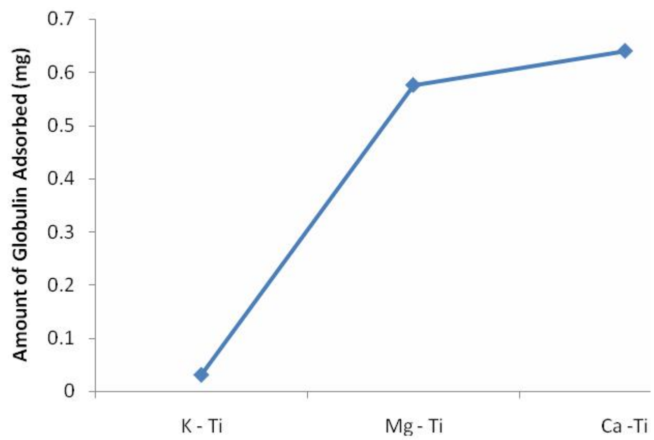

Figure 1 : Effect of cation on the adsorption of globulin onto titanium

No significant difference was observed in the amount of globulin adsorbed to untreated titanium at pH 3.0 and pH 7.0. However pretreatment of titanium

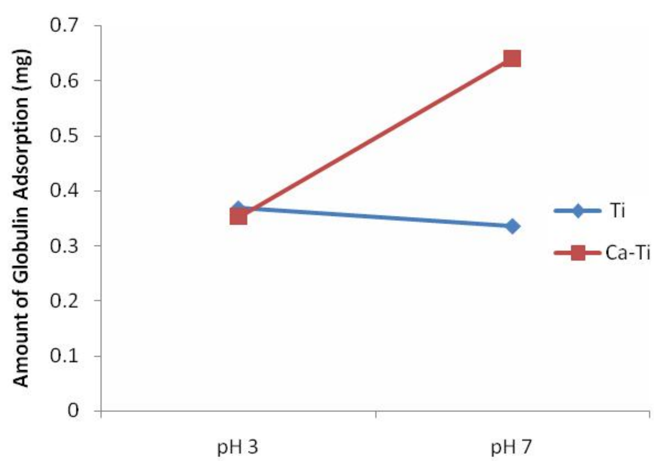

Figure 2 : Effect of pH on the adsorption of globulin onto titanium

with calcium at pH 7.0 led to increased in the adsorption of globulin significant as illustrated in Figure 2.

Considering the result in Figure 3, the effect of incubation time on the adsorption of globulin to Ca treated Ti and untreated Ti from the results obtained. It was observed that most of the globulin became adsorbed to Ti powder within 1hr of implantation for Ca treated sample while for the untreated Ti lower amount of globulin was adsorbed in the first 10mins. The amount of globulin adsorbed became constant at 30mins of implantation as observed from Figure 3., for both the Ca treated and untreated Ti samples.

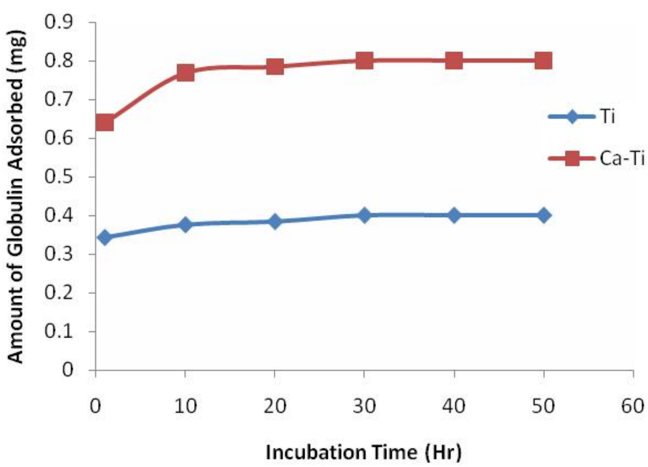

Figure 3

DISCUSSION

In these studies it was observed that variation in cations, incubation time and pH of an environment around an implanted titanium metal influence its adsorption to globulin, and hence titanium metal used as implant in patient could bind calcium more than cations. The overall effect of cations as shown in figure 3 could be due to the fact that when titanium metal implant comes in contact with proteins in presence of cations will create a competition for them as regard binding to the site on TiO_2. So Mg^{2+} has less ability to react with calcium binding such as globulin than calcium and also present in lower concentration. Other cations such as zinc, potassium, cadmium will not present any serious competition in a patient[12]. More so this studies also indicates that Ca^{2+} and Mg^{2+} acts through a non-specific attraction that can be linked to their electrovalencies. So it can be probably said that the above noticed effect of cation is due to electrostatic interaction[12].

Since it is well established that calcium treated titanium used as implant and exposed to body fluid might increase its biocompatibility with bones and induce a subsequent adsorption of calcium binding macromolecules on implant surface[8], then the result in figure 4 shows at pH 7.0, the negatively charge globulin and the TiO_2 surface allowed for the binding action of divalent cation while at pH 3.0 globulin is positively charge and the negatively charged $Ti0_2$ surface is neutralized owing to a shift in its Zeta potential from -32.6 at pH 7.15 to 3.0 at pH 4.0[11,16]. Therefore eliminating the effect of Ca^{2+} in the adsorption process, and the change in zeta potential of titanium could also explain the similar adsorption of globulin to untreated under different pH conditions as shown in figure 4 whereby both components are positively charged at pH 3.0 and negatively charge at pH 7.0. A charge in binding properties of proteins might also be explained by an altered conformation upon drastic pH charges[7,11,15,20].

Looking at Figure 2 above, it will be observed that a positing excise between the extent of globulin adsorption process on titanium samples treated with calcium and the mechanism of the adsorption process could be attributed to electrostatic attraction that depends on electrovalence of the ions. Our result also suggested that the amount of globulin adsorbed was higher at physiological pH. So the electrostatic attraction dictates the main mechanism of adsorption of globulin to titanium process at physiological pH (7.0 - 7.4) making more globulin to bind Ca^{2+} to its electrostatic sites such that the ion serve as a legend between the globulin and the titanium surface[12].

CONCLUSION

From the results obtained it can be concluded that divalent cations such as Mg^{2+}, and Ca^{2+} were seen to affect the adsorption of globulin onto titanium significantly. So the binding of globulin to titanium treated with Mg^{2+}, and Ca^{2+} were observed to be much higher than K^{+} treated as evident from the studies. Hence the mechanism of the adsorption process can be attributed to electrostatic attraction that depend on electrovalency of these ion. However the amount of globulin adsorbed is higher at physiological pH and the presence of calcium in blood serum of a patient and physiological condition (pH 7.0-7.4) make this results relevant to the mechanism of the binding of globulin onto titanium during implantation in vivo.

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