Brazilian J Med Biol Res (1994) 27: 2569-2571

ISSN 0100-879X
Short Communication

The interaction of blood proteins with alpha-alumina

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The use of alumina (α-Al_2O_3) as a material for cardiovascular applications was investigated on the basis of protein adsorption and thrombus formation on the material. The adsorption of ^{125}I-labelled albumin and fibrinogen from phosphate buffered saline (pH 7.35, 0.100 M NaCl, 8.66 mM KH_2PO_4 and 41 mM Na_2HPO_4) solution on ceramic discs of alumina was studied. Both albumin and fibrinogen presented affinity for ceramic surfaces, with adsorptions of 1.47 ± 0.06 ng/cm^2 and 0.198 ± 0.01 ng/cm^2, respectively. Scanning electron micrographs of the α-Al_2O_3 surfaces after contact of the discs with whole human blood showed a thrombogenic behavior of alumina alpha. These results indicate a hemoincompatible property. Although critical surface tension (γ_C: 21.8 dynes/cm) of the disc surfaces determined by contact angle technique of sessile drops indicates that alumina alpha is a biocompatible material, by this criterion, the data reported here indicate that α-Al_2O_3 cannot be used for cardiovascular applications.

Key words: bioceramics, biomaterial, thrombogenic properties, alumina.

Bioceramics are ceramic materials with a specific physiological behavior. The inert ceramics such as alpha-alumina and calcium phosphate undergo little or no chemical change during long-term exposure to the physiological environment. Most investigations related to bioceramics refer to them as potential material for the construction of orthopedic and oral prostheses (1,2). Ceramic materials have not been extensively tested in the cardiovascular system and the

Publication supported by FAPESP.
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initial events exposing them to blood have not been studied in detail (3). It is known that ceramics introduced into blood environment can cause problems of surface-induced thrombosis.

Proteins at solid-liquid interfaces play important roles in a variety of biological processes. Thus, the nature of blood proteins adsorbed onto biomaterials can be used to determine the hemocompatibility of the surface (4).

In the present study contact angle measurements used to determine the critical surface tension of alumina alpha as well as albumin- and fibrinogen-labelling to study plasma protein adsorption were carried out. Platelet adhesion to the surface was studied by scanning electron microscopy (SEM).

Aluminum oxide powder (α-Al_2O_3) was supplied by Alcoa (A-16 SG). The powder was compacted into discs 12 mm in diameter under a pressure of 120 MPa and sintered at 1650°C in the presence of air for 1 h. SEM of the ceramic discs showed a smooth surface after the sinterization process. The α-Al_2O_3 discs were cleaned with neutral detergent followed by extensive rinsing in distilled water and washing in acetone with an ultrasonic cleaner. The samples were vacuum dried at room temperature. Contact angle measurements were made using sessile drops of water (γ_{LV}: 73.12 dynes/cm), glycerol (γ_{LV}: 63.4 dynes/cm) and octane (γ_{LV}: 21.8 dynes/cm). Critical surface tension, γ_C, of the ceramic surface was obtained by plotting cos θ against γ_{LV} and extrapolating to the value of γ_{LV} corresponding to cos $\theta = 1$ (5).

In order to quantify the surface concentrations of albumin (BSA) and fibrinogen adhering to the ceramic samples, the proteins were labelled with ^{125}I using the chloramine T method (6). The adsorption of labelled proteins was measured after 2-h equilibration at 37°C in an appropriate cell. Unadsorbed protein was rinsed off with phosphate buffered saline (PBS, pH 7.4). Surface protein adsorption was determined by counting the radioactivity in a gamma counter.

Blood compatibility of the ceramic discs was evaluated by *in vitro* tests. The platelets adhering to the surfaces of four alumina discs were counted after whole human blood contact. Analysis was carried out by SEM microphotography. The average number of adhered platelets was obtained from five photographs of the different surface areas (2.26 cm^2 each) of the same sample.

When a material is placed in contact with blood, the first event to occur is the adsorption of proteins onto the surface, followed by platelet adhesion and activation. Surface-induced platelet activation is largely dictated by the type and amount of blood proteins adsorbed at the biomaterial/blood interface (7). Adsorption of fibrinogen is known to accelerate platelet adhesion and activation. On the other hand, albumin adsorption on the synthetic surfaces can inhibit platelet activation, thus preventing clot formation (8).

In our experiments, both albumin and fibrinogen were bound significantly to ceramic surfaces. The adsorption of albumin was 1.47 ± 0.06 ng/cm^2 and the adsorption of fibrinogen was 0.198 ± 0.01 ng/cm^2. However, the ceramic affinity for albumin was not sufficient to inhibit platelet adhesion. Scanning electron micrographs of the ceramic surfaces after contact with blood showed that α-Al_2O_3 presented thrombogenic activity. The total number of adhered platelets on each disc was large, 120 ± 20 per disc, and a large quantity of adhering fibrin was also observed.

The critical surface tension determined for α-Al_2O_3 surfaces was γ_C: 21.8 dynes/cm. According to Bayer (9), this value indicates that α-alumina is a biocompatible material which can have pratical applications for the construction of prostheses in orthopedics and dentistry. However, on the basis of data presented here α-Al_2O_3 cannot be used in cardiovascular applications without some form of surface modification.

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Received May 4, 1994
Accepted September 9, 1994