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Comparative Study of Proliferation of Bovine Albumin Serum on Different Metallic Substrates for Biomedical Applications

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Abstract: Pure titanium (Ti), titanium alloys, and different types of stainless steel are often employed for applications in orthopedic implants. Among these, titanium is considered the "gold standard." However, tissue reactions around these metal surfaces and the changing trend to leave orthopedic devices in the body have led to a new examination of the preferred material. Nowadays various metallic materials are commonly employed to improve the life time of bioimplants. Three types of metals (SS-316L, SS-304L and pure Titanium) were used for a comparative study by considering adsorption properties of all. Metallic materials are attractive due to properties like less coefficient friction, high hardness value, and chemically inert in nature which make them attractive to be used in biochemical devices. The surface of these metals was prepared to study the adhesion of different type of animal proteins adhesion on their surface. Protein growth on these materials was studied by using AFM (atomic force microscope), SEM and FTIR at different time intervals (24h, 48h, 72h, 96h). The difference in density and weight of the samples before and after the adsorption of protein on substrates confirms the proliferation process on metals. Promising results were obtained at time interval of 96 hours. Overall, all the samples show similar behavior.

Keywords: Biocompatibility, Bovine Serum, Comparative Study, Metallic Materials

1. Introduction

Almost 80% of implants are developed by using metals due to their incomparable mechanical properties, resistance to corrosion and acceptable biocompatibility. The main metals which are biocompatible and corrosion- resistant are stainless steel (SS), cobalt-chromium alloy and titanium and its alloys.

Metallic implants are in contact with blood that contains not only inorganic ions but also organic molecules, mainly proteins which can be affected by the corrosion process of metals. In case of biomaterials, it is normally accepted that one of the initial things which considerably affect the biocompatibility of implants is prompt adsorption phenomenon of proteins from biological fluids onto biomaterial surfaces because it is the early step of thrombosis, foreign body reaction and cell adhesion [1-3]. Among the factors which determine the success or failure of implants, surface properties of the material, which determine the nature of the protein and the number of cells adhered on the surface at the time of implantation, are more important. Proteins accumulate at interfaces, a property that can be both a practical asset and a problem. Cellular attachment concerns several features that include the cell-surface behavior, nature of bio-surface materials concerning hydrophobicity, charge, roughness, softness and surface chemical composition [4]. The osseointegration process is a success of an implant, so the surface of implant should allow passable cell adhesion, proliferation to generate the formation of healthy bone at interface. One of the strategies to enhance the biocompatibility and generate osteogenesis to prevent infection is by treating the surface of the implant material

[5-10]. Therefore, in physiologic environment, adsorbed serum proteins play important role in the event. When an implant is place in a medium, the outer environment will immediately cover the surface with biological fluids. Thus, in vivo and in vitro studies, cells do not interact with the original implant surface, but with the layer of protein on its surface [11]. This protein layer then mediates the interaction of the implant material with the cells arriving from the surrounding tissue [12]. Thus, protein adsorption onto metallic surfaces affects the biocompatibility of the materials. Most common metals like titanium, some grades of stainless steel like 316 or 304, titanium oxide, titanium nitride, cobalt–chromium alloy, and nickel alloys have been used for different biomedical applications. In vitro, rigid surfaces like oxide-covered titanium adsorb proteins within seconds of exposure to blood plasma while stainless steel and CoCr implants are not considered an ideal choice for cementless implants. The adsorption of proteins on the metal surface occurs through physical adsorption of the OH group on the surface by different types of bonding [13-16]. An enhancement of protein adsorption encourages cell adhesion and growth and thus causing rapid bone growth and osteointegration at the implant surface. But in case of biomaterials, protein adsorption is much less desirable due to adverse responses of host materials like coagulation of blood and inflammation.

However, in biomaterial field, protein adsorption is much less desirable because it can cause adverse host responses such as blood coagulation and complement activation. On the other hand, cell adhesion to surfaces depends on the availability of specific protein-binding sites. The desire to control, predict, and manipulate protein adsorption has been the main driving force for past research in this field. This has led to a plenty of current research directed at gaining a better understanding of the behavior of proteins at interfaces, which is reflected in the large number (>300) of papers on the subject published during the past year [17]. Different studies indicate that the cause of many hazards in the body is the unwanted behavior of adsorbed proteins on prosthetic surfaces exerting conformational changes subsequently triggering adverse effects. Bovine Serum Albumin (BSA) is of particular interest when investigating the interaction of proteins with implants, and it is regarded as a model protein. Sumaira et al [18] investigated the protein adhesion on PVD coated stainless steel samples by Scanning Electron and AFM studies. Burstein and Liu [19] examined the properties of bovine serum on the nucleation of corrosion pits on stainless steel (316 L) and pure titanium metal in Ringer's physiological solution at 37 °C. Lundin et al (10) demonstrated that protein increases the metal dissolution and that the metal release mechanism does not depend on SS grades. The motivation of current topic is the comparison of different metallic substrates for the proliferation of bovine serum albumin for a specific period of time. Pure titanium metal and two grades of stainless-steel SS 304 and 316L were used for this study. These grades are most commonly used in indigenous surgical industry of Pakistan.

2. Experimental Work

Three types of different metallic substrates (SS304, SS 316L and pure titanium) were selected for the study. The chemical composition of the substrate metals was confirmed by Optical Emission Spectrometer (Metal Lab 75-80J). After the chemical analysis of metals, their surface was prepared. The samples were fine grinded and polished till 1μm by using the diamond paste. The polished surfaces were then checked for their roughness analysis. For this, Surface profilometer (SURFCORDER) was used for roughness analysis. After obtaining the required roughness, samples were cleaned in different media in order to remove any dust or contaminants from the surface before soaking process. Initially the samples were placed in ultrasonic cleaning bath containing carbon tetra chloride solution. Temperature of bath was maintained at 30°C and cleaning was carried out for approximately half an hour. CCl_4 removes all the greasy material stuck to the surface. After this, the samples were thoroughly washed with double distilled water. Same process was repeated by using acetone in the bath to remove any dust and contaminants from the surface of samples. For in vitro testing, Bovine Albumin Serum was purchased from Sigma Aldrich, 100 ml protein solution (Bovine Albumin) was prepared by taking 5mg of bovine albumin in 100 ml of double distilled water in a sterile beaker and prepared samples were soaked in it for 24 hours at 4 degrees centigrade in controlled conditions according to in vitro method"/EN 30993-5. Samples were washed with a 90% methanol solution, 8% de-ionized water and 2 % glacial acetic acid. Then amount of protein adsorbed on the surface was calculated for 12 hours, 24 hours, 48 hours and 96 hours respectively. Samples were evaluated for cell adhesion using atomic force microscope (AFM). Scanning Probe Microscope (SPM) CP-II was used for the AFM analysis to check the adhesion of protein on the surface. The analysis was carried out in the contact mode and area of 10 by 10 μm 2 was scanned during AFM analysis in the topography mode. The scanning rate was maintained at 1Hz and the set point value was adjusted at 13 nN. All scans were taken in X direction and the gain parameter was adjusted at 0.35.

3. Results and Discussions

3.1. Physical Properties

Table 1 shows chemical composition of substrates performed on emission spectrometer which appears to be AISI 304-L, AISI 316-L and pure Titanium Metal. Table 2, 3 and 4 shows the weight and density of prepared samples before and after dipping.

Table 1. *Chemical Composition of substrate materials.*

Sr.#	Material	Carbon	Manganese	Phosphorus	Sulfur	Silicon	Nickel	Chromium	Molybdenum
1	SS 304 L (A)	0.012	1.021	0.029	0.004	0.468	8.084	18.792	-
2	316 L (B)	0.027	2.017	0.027	0.014	0.567	10-115	16.451	2.101
3	Titanium	99.200% pure							

Table 2. *Weight and Density of SS-304L samples.*

Sr.No.	ID of samples	Weight in grams (before dipping)	Weight in grams (after 24 hours)	Weight in grams (after 48 hours)	Weight in grams (after 72 hours)	Weight in grams (after 96 hours)
1	A-A4	9.001	9.249	9.672	9.923	10.101
2	B-B4	10.386	10.679	10.991	11.009	11.191
3	C-C4	17.128	17.464	17.818	17.901	17.981
4	D-D4	11.604	11.901	12.210	12.292	12.325
5	E-E4	11.121	11.625	11.925	12.004	12.082

Table 3. *Weight and Density of SS-316L samples.*

Sr.No.	ID of samples	Weight in grams (before dipping)	Weight in grams (after 24 hours)	Weight in grams (after 48 hours)	Weight in grams (after 72 hours)	Weight in grams (after 96 hours)
1	A-A4	11.897	11.910	11.970	12.201	12.240
2	B-B4	12.228	12.468	12.772	12.919	12.991
3	C-C4	8.985	9.012	9.266	10.403	10.413
4	D-D4	9.252	9.452	9.818	10.125	10.201
5	E-E4	11.714	11.909	12.320	12.372	12.412

Table 4. *Weight and Density of Titanium samples.*

Sr.No.	ID of samples	Weight in grams (before dipping)	Weight in grams (after 24 hours)	Weight in grams (after 48 hours)	Weight in grams (after 72 hours)	Weight in grams (after 96 hours)
1	A-A4	11.924 (A)	11.949 (A1)	12.012 (A2)	12.172 (A3)	12.177 (A4)
2	B-B4	13.442 (B)	13.801 (B1)	13.886 (B2)	14.019 (B3)	14.119 (B4)
3	C-C4	9.490 (C)	9.499 (C1)	9.890 (C2)	10.106 (C3)	10.207 (C4)
4	D-D4	16.788 (D)	16.901 (D1)	16.921 (D2)	17.100 (D3)	17.191 (D4)
5	E-E4	12.001 (E)	12.420 (E1)	12.860 (E2)	12.892 (E3)	12.925 (E4)

3.2. Scanning Electron Microscopy

The morphological studies of the samples were performed by Scanning Electron Microscope (Hitachi). The aim of this study was to observe adsorption of protein on titanium plates. Micrographs of scanning electron microscopy of adsorbed protein on titanium surface is illustrated by Figure 1 A to A4). Figure represents adsorption of protein having extremely uneven surface. However subsequent to increase in time period, adsorption tends to improve in a slow manner as in Figure 1 (A1, A2, A3, A4). In case of 304L steel (Figure 2), the adsorption is considerably improved with the time in an uneven manner through-out the surface. Abrupt type of adsorption is shown in SS 316L substrate as in Figure 3 (A1-A40).

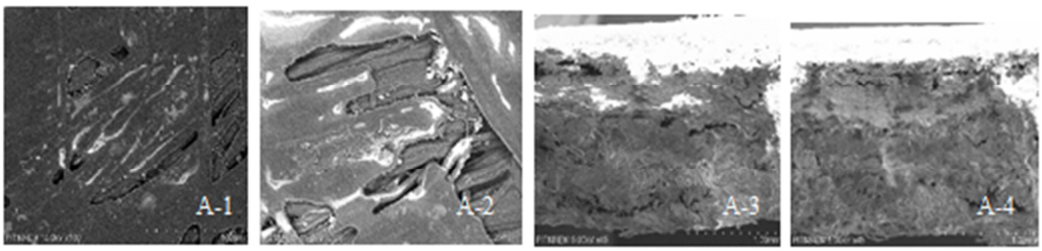

Figure 1. *SEM images of Titanium Metal after adsorption.*

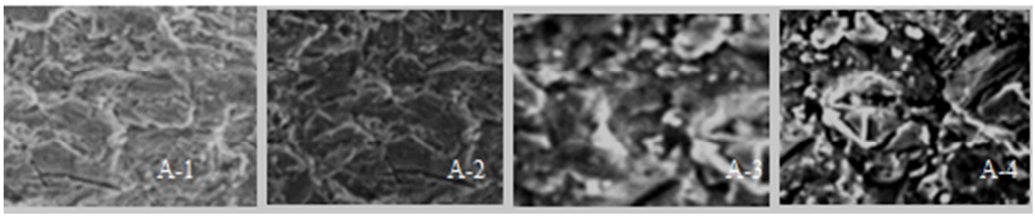

Figure 2. *SEM images of SS-316L after adsorption.*

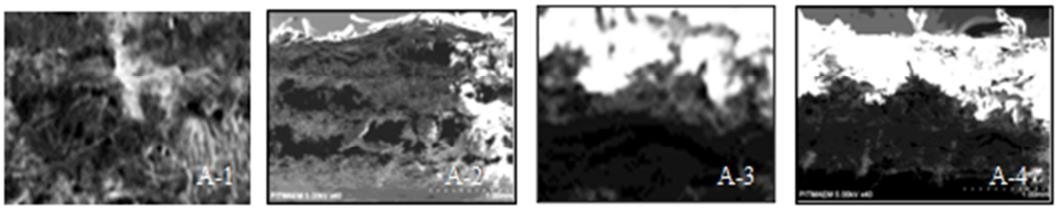

Figure 3. *SEM images of SS-304L after adsorption.*

3.3. Atomic Force Microscopy

Adsorption of Bovine Albumin Serum on titanium metal was 75µg/cm^2 showing more absorbance as compared to other metals like steel. The bright portions in figure depict the presence of protein on the coated surface, which is also confirmed by 3D images as well.

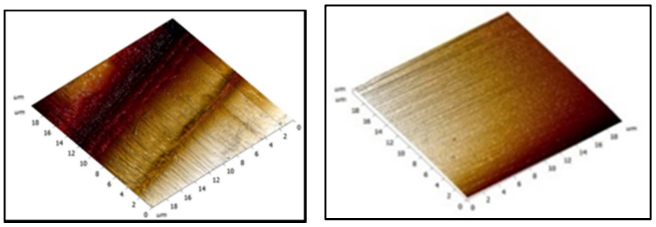

Figure 4. *AFM image of adsorbed protein on Titanium.*

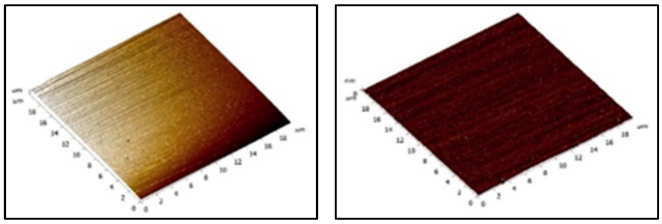

Figure 5. *AFM image of adsorbed protein on 316L.*

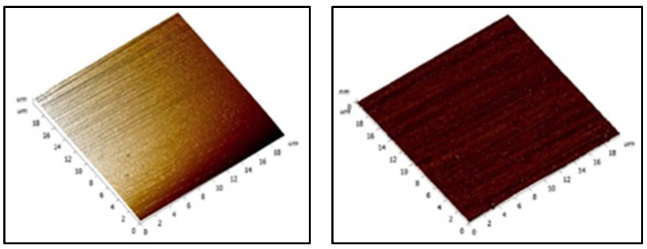

Figure 6. *AFM image of adsorbed protein on 304L.*

4. Conclusion

In the case of orthopedic biomaterials, the interaction between cells and their substrates can influence the nature of the bone-implant interface, Nowadays, biocompatible implants for internal treatment have been developed to avoid the need of a surgery for device removal. Therefore, to reduce the potential risks of biocompatible metallic implants, like corrosion, stress protection weakening of bone, these implants have been considered as a good alternative of conventional materials which, in turn, will determine the long-term stability of the implant prostheses.

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