Comparative performance of Nitinol surfaces in protein adsorption and platelet adhesion. Preliminary results.

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Abstract. Nitinol superelasticity can be favorably used to design self-expending medical devices that can reduce the level of vessel injury associated with neointimal proliferation and thrombosis. This study was performed to compare the relative thrombogenicity of Nitinol surfaces prepared using various protocols (polished 600 grit and 1 μm finish, chemically-etched, heat treated and electropolished in two different electrolytes). Albumin (A) and fibrinogen (F) adsorptions from single solutions were examined with radiolabeling. Human platelet adhesion, spreading, and aggregation were studied by scanning electron microscope. Principal findings were: protein adsorption and platelet responses are strongly dependent upon material surface chemistry. Rougher substrates and thicker surface oxides bound higher amounts of total proteins. Within Nitinol groups, fibrinogen adsorption varied from 130 to 300 ng/cm^2, and that of albumin - from 30 to 130 ng/cm^2, and the A/F ratio from 0.15 to 0.6. Disregarding differences in surface morphology, the amounts of adsorbed fibrinogen was in direct proportion with the titanium surface concentration. In contrast, adsorption of albumin and the albumin/fibrinogen ratios correlated with the Ni surface concentrations. Lower albumin adsorption from a single protein solution corresponded to significantly higher platelet deposition and total surface coverage by the base layer of fully spread platelets. Nitinol surfaces electropolished in different electrolytes showed a two-fold difference in the amount of adsorbed albumin. Heat treatment of Nitinol surfaces reduced the number of adhered platelets. Formation of thrombuslike platelet clusters was obvious in the case of 600 grit finish and one of the heat treated surfaces. Unexpectedly better hemocompatibility of Nitinol surfaces with the elevated Ni content (4-7%) is discussed in respect to possible material toxicity, based on parallel studies of Ni release, lymphocyte and endothelial cell proliferation. Surfaces slightly enriched in Ni might be an option to promote better hemocompatibility without compromising biocompatibility of Nitinol.

Figure 1. Schematic depiction of platelet spreading divided into five shape categories. From left to right, these stages of platelet spreading are defined as follows: round (R) or discoid: no pseudopodia present; dendritic (D) or early pseudopodial: one or more pseudopodia with no evident flattening; spread dendritic (SD) or intermediate pseudopodial: one more pseudopodia flattened, hyaloplasm not spread between pseudopodia; spreading (S): hyaloplasm spread between pseudopodia; and fully spread (FS): hyaloplasm extensively spread, no distinct pseudopodia (Goodman S. 1999. Sheep, pig, and human platelet-material interactions with model cardiovascular biomaterials. J Biomed Mater Res 45, 240-250).

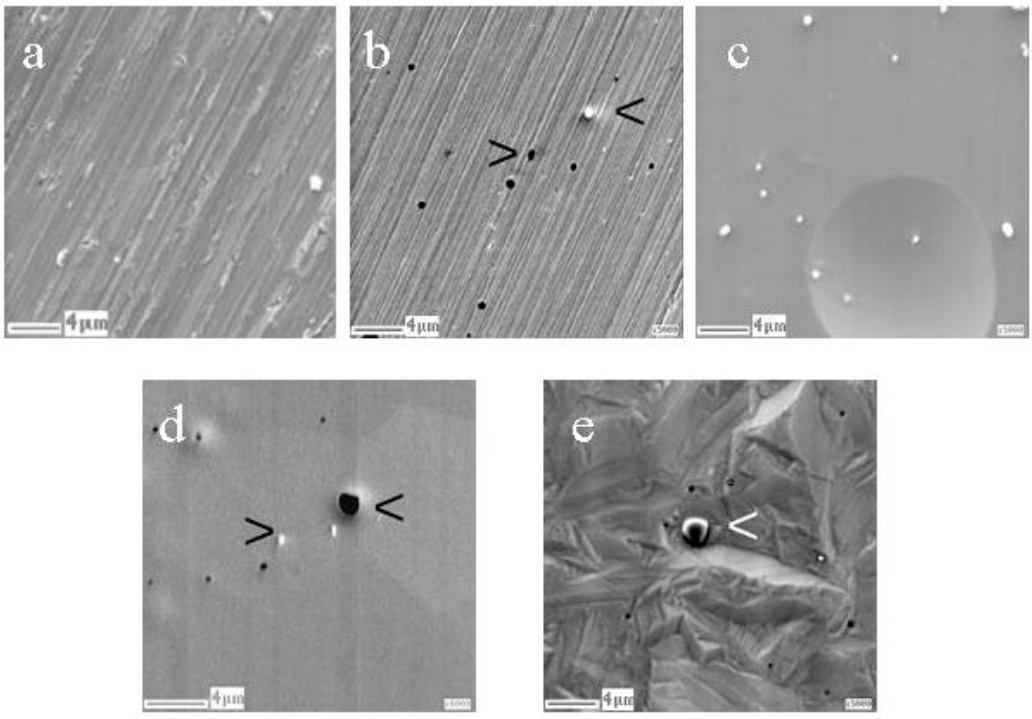

Figure 2. Representative SEM images of Nitinol surfaces. (a) mechanically polished 600 grit finish (Mp); (b) mechanically polished 1 μm finish (Mp1μm): inclusions like titanium dioxide protruding through the surface (white particle) and Ti based carbides or carbonates (black particles) became evident on smoother surfaces; (c) chemically etched: particles of titanium dioxides are protruding through the surface, no carbon-containing particles were detected; (d) electropolished in the room temperature electrolyte (Ep1) in the austenitic phase: smooth surface with embedded inclusions; (e) electropolished in the low temperature electrolyte in the martensitic phase (Ep2): surface relief resulting from the reverse martensitic transformation upon heating to room temperature can be seen, as well as embedded carbon-containing particles.

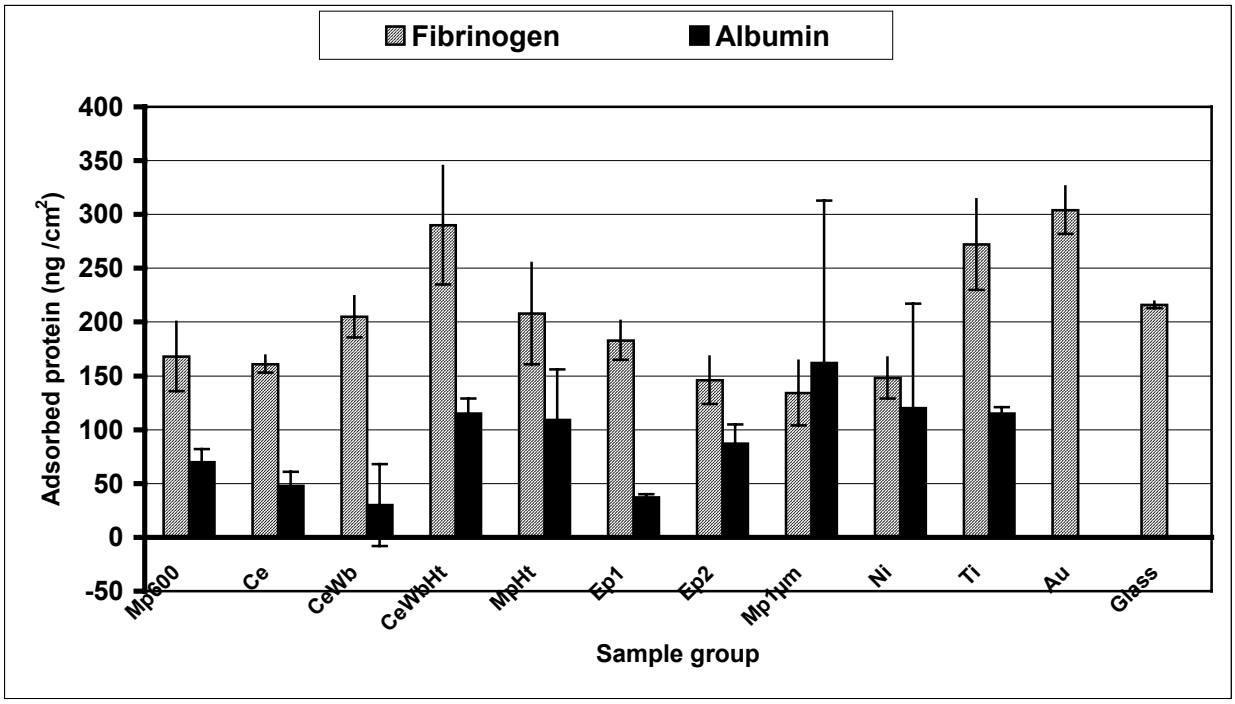

Figure 3. ^{125}I - labeled fibrinogen and albumin adsorption on Nitinol, pure nickel, titanium, gold, and glass surfaces. Mp – mechanically polished 600 grit finish, Ce – chemically-etched, CeWb – chemically etched and aged in boiling water, ChWbHt – additionally heat treated at 500^{o}C for 15 min in air, MpHt-mechanically polished 600 grit finish and heat treated, Ep1 and Ep2 –electropolished in austenite and martensite, respectively; Mp1μm - mechanically polished.

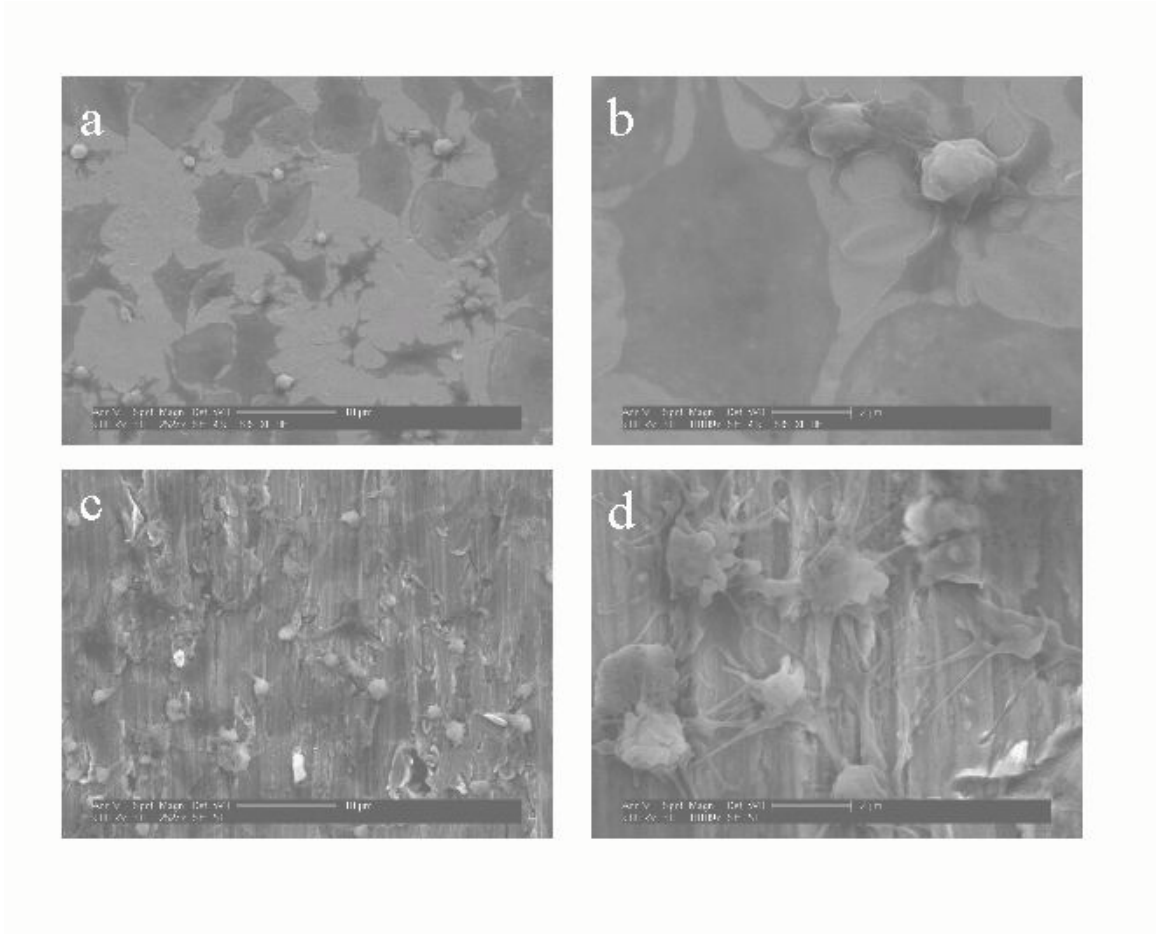

Figure 4. Scanning electron micrographs of human platelets adhered to electropolished Ni (images a and b with x2500 and x10000 magnifications, respectively) and acid etched Ti surfaces (c and d with x2500 and x10000 magnifications, respectively). Platelets on Ni surface present mostly in the fully spread state forming a thin monolayer (a,b). Significantly less platelets are observed on Ni surfaces in the intermediate stage of spreading SD-S. On acid etched rough Ti surfaces, platelets are spread poorly. A large number of pseudopodial platelets in dendritic (D) and spread dendritic (SD) stages is evident.

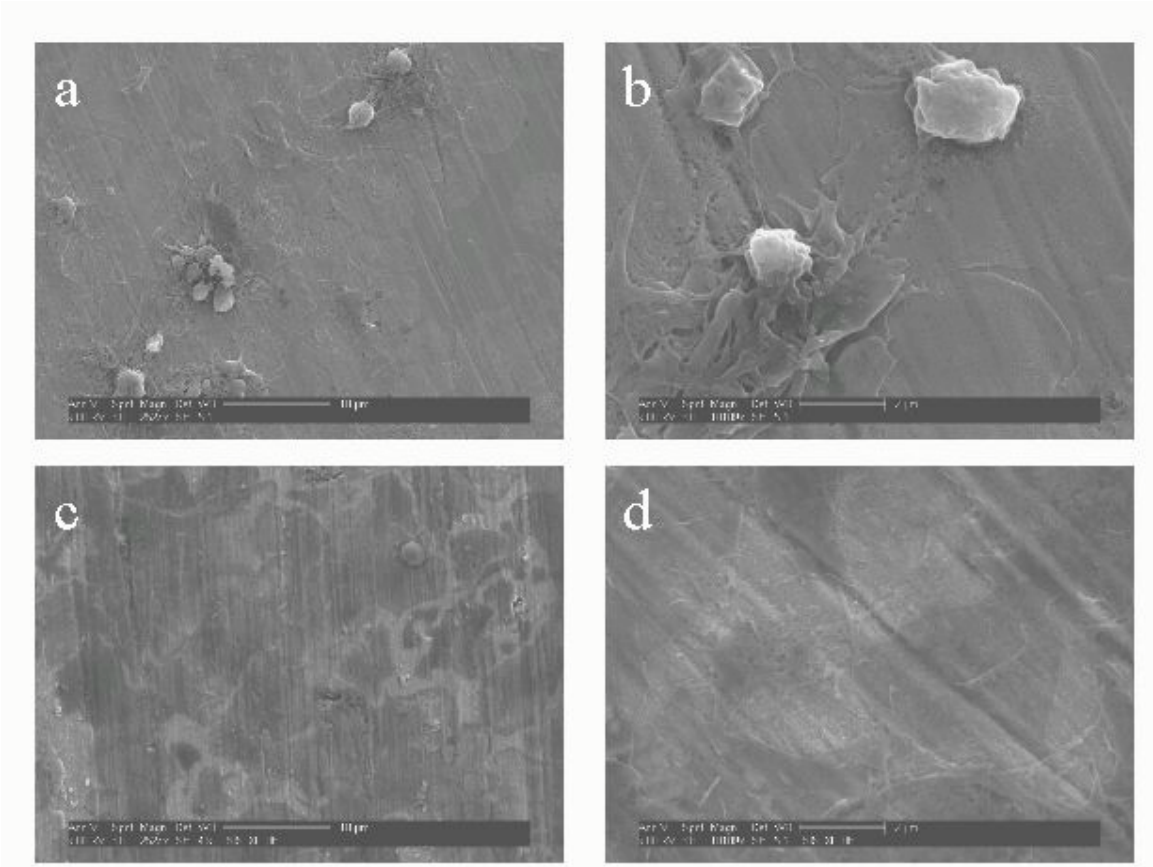

Figure 5. Scanning electron micrographs of platelets adhered to Mp 600 grit finish (a,b) and MpHt (c,d) Nitinol surfaces. Magnifications are similar to Figure 4. Platelet aggregation in thrombus like structures (a) on Mp 600 surface is due to uneven platelet spreading evident from the image (b). Virtually all platelets are well spread on the surfaces of heat treated samples.

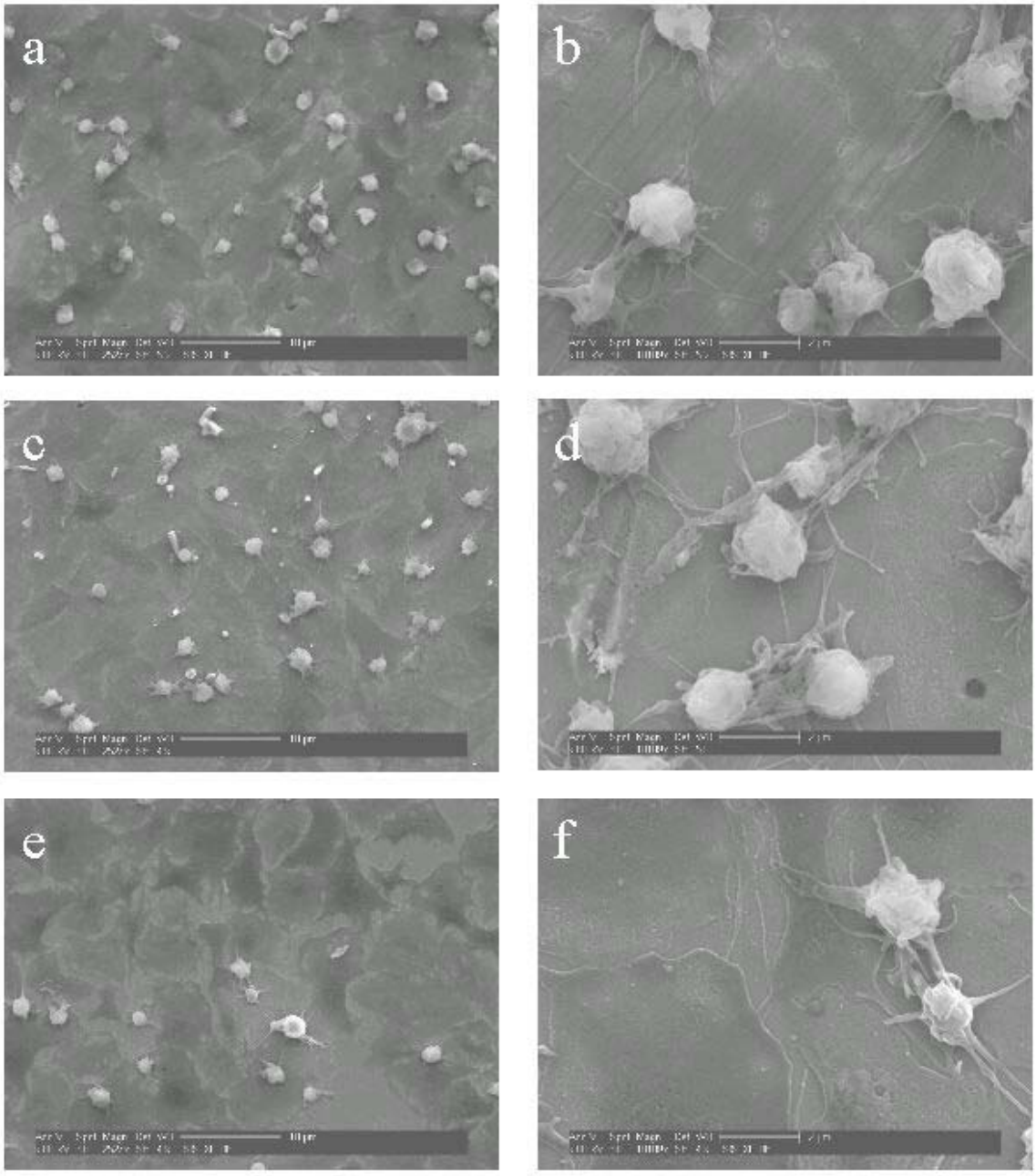

Figure 6. Scanning electron micrographs of platelets adhered to Mp1μm finish (a,b), electropolished (Ep1) in austenite at room temperature (c,d), and electropolished (Ep2) in martensite (e,f) surfaces of Nitinol. Adhered fully spread platelets form a base layer (monolayer). Surface coverage by fully spread platelets is similar for these three groups of samples. Pseudopodial platelets adhered to the base layer are on the stage of spreading intermediate between dendritic and spread dendritic. Occasionally, platelets could form clusters up to seven cells, (a) due to cohesion of pseudopods (b,d, and f). A sticky base layer poorly adhered to the substrate is wrapping pseudopodial platelets on the surface of an Ep1 sample (d). Needle-like white particles of titanium dioxides (Nitinol inclusions) can be seen on the surface (image c,e). The numbers of adhered pseudopodial platelets decrease in the following sequence of surface treatments Mp1μm > Ep1 > Ep2.

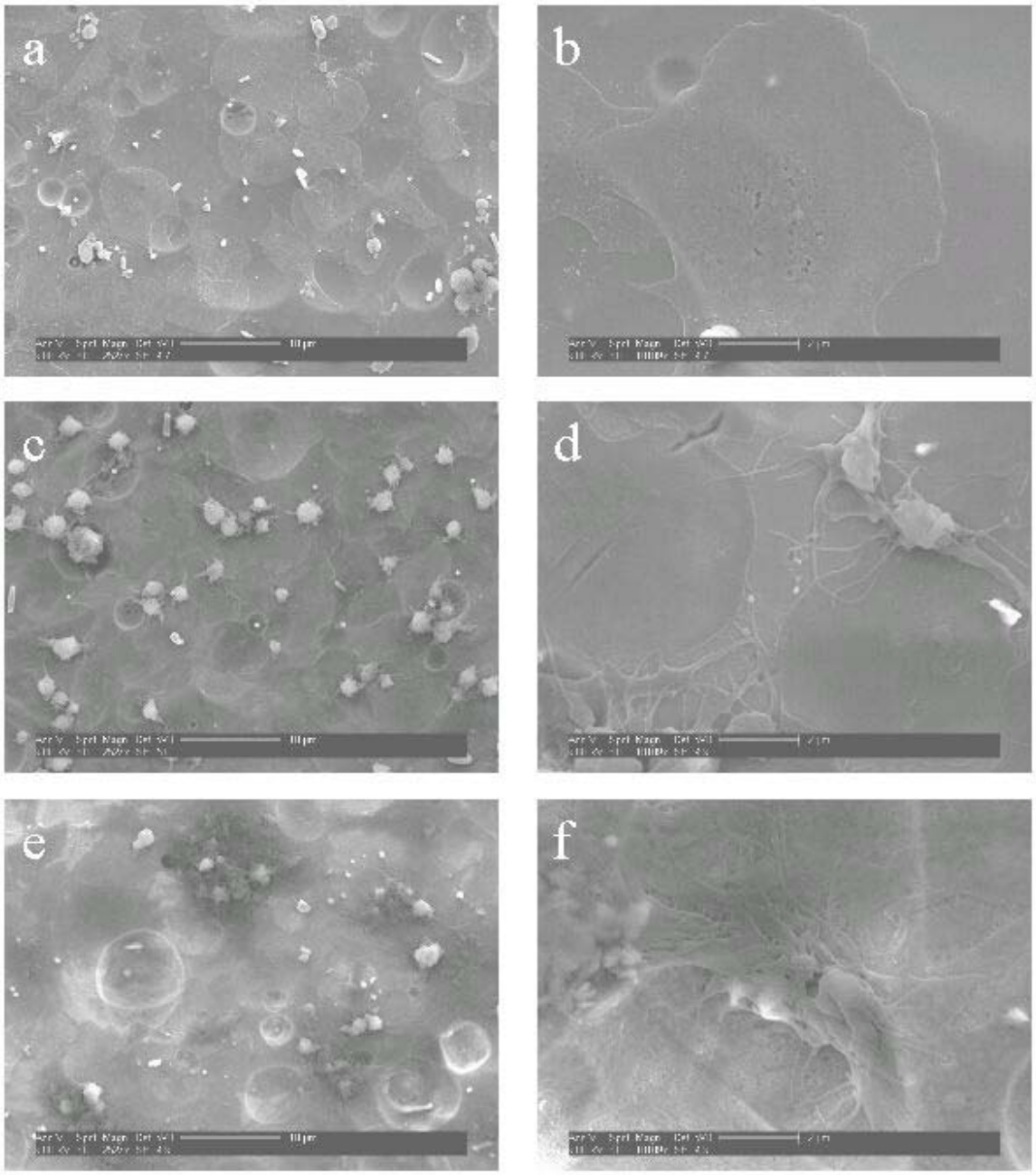

Figure 7. Scanning electron micrographs of platelets adhered to: (a,b) chemically-etched, Ce, (c,d) additionally boiled in water, CeWb, and (e,f) heat treated (CeWbHt) Nitinol surfaces. Significantly lower surface coverage by fully spread cells compared to other Nitinol surfaces is evident, especially in the case of Ce surfaces < 50% (images a,b). The base layer consists of a monolayer of fully spread cells in the case of Ce (b) and CeWb surfaces (d). In contrast, platelets, forming base layer on heat treated surfaces, did not spread evenly (f). Two-dimensional cell (SD-D stages of spreading) clusters can be observed on chemically-etched (a) and boiled in water Nitinol surfaces (c), and thrombus-like structures are evident on heat treated surfaces (e).

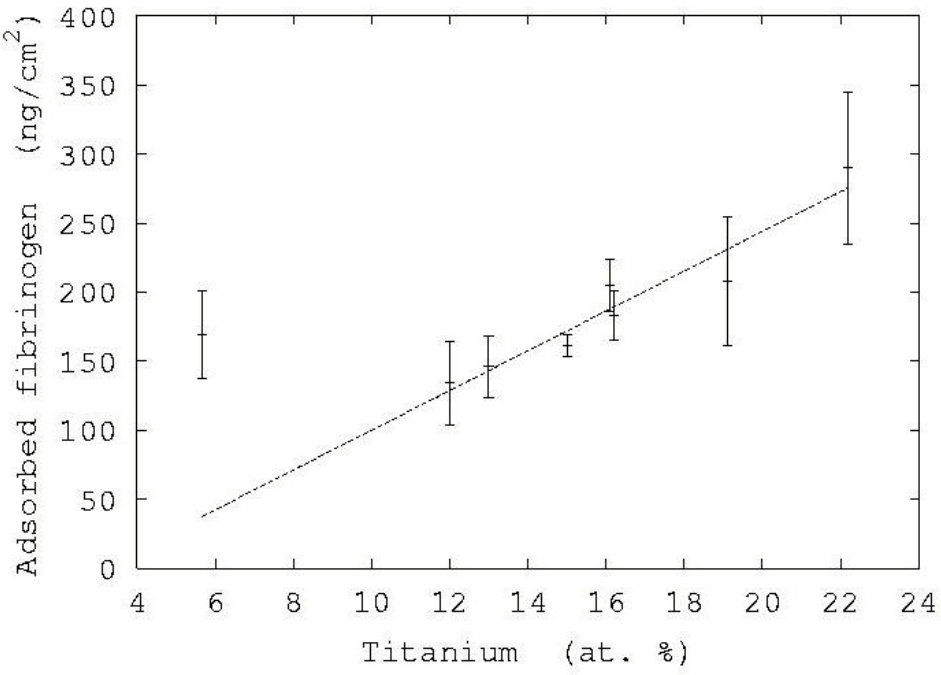

Figure 8. Fibrinogen adsorption from a single protein solution versus titanium concentration on the studied Nitinol surfaces. All data, with the exception of the 600 grit finish surface, can be approximated by one line, indicating direct proportionality between fibrinogen adsorption and Ti surface concentration.

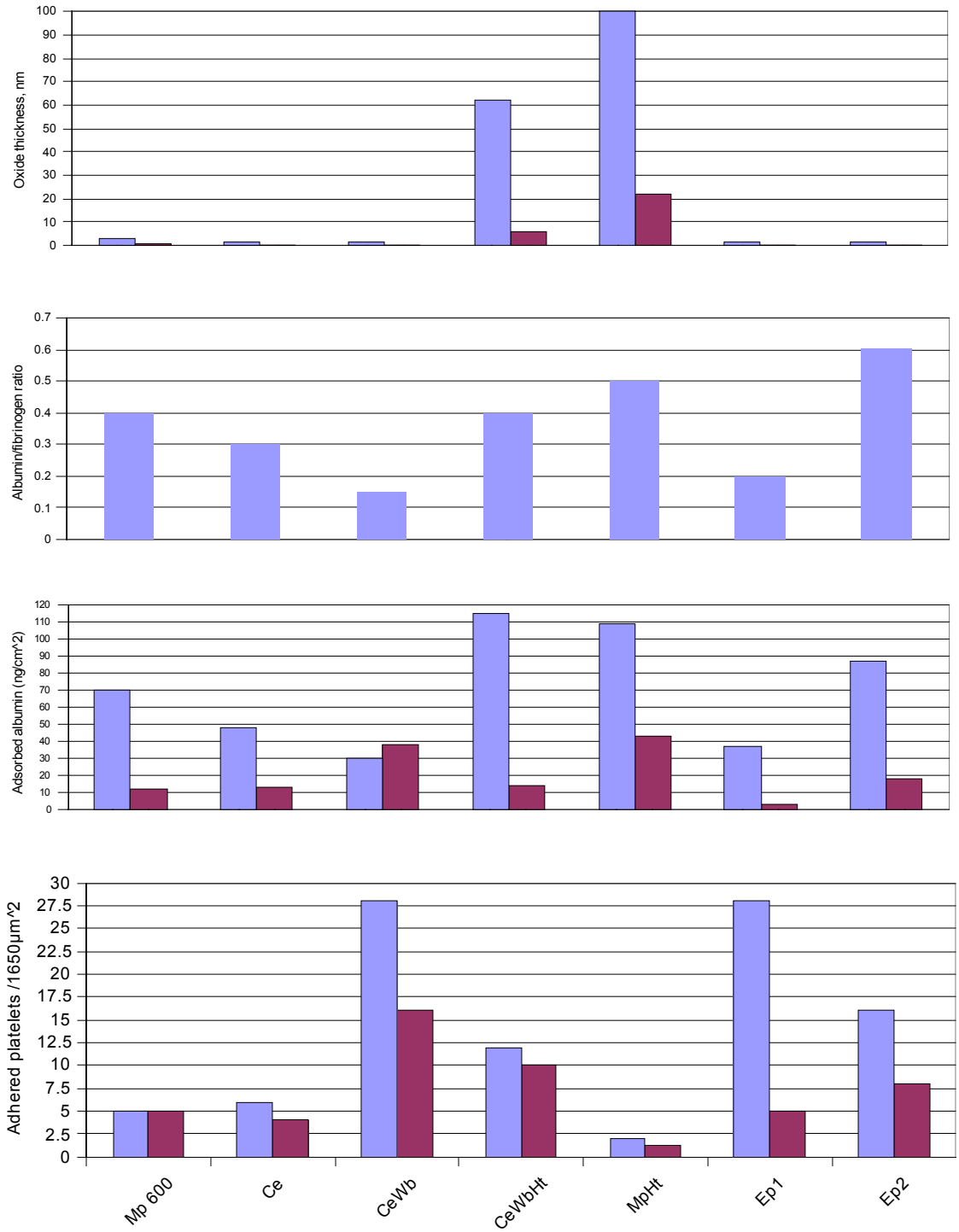

Figure 9. The correlation between adsorption of albumin (c), albumin/fibrinogen ratios (b), and surface oxide thickness (a), and adhesion of platelets to a base layer (d). The number of adhered platelets is the inverse proportion to the amounts of albumin adsorbed to Nitinol surfaces.