INTRODUCTION: Elevated concentrations of circulating metal derived from orthopedic implant degradation may have direct and accumulatory immunologic implications over the long term.\(^1\) Previously, elevated levels of Cr, Co and Ti have been reported in the serum of patients with both well functioning and failed total joint replacements.\(^2,\)\(^3\) Differences in the bioreactivity of specific metal-implant degradation products are related to metal-protein binding. However, little is known about the short and long term pharmacodynamics and bioavailability of circulating metal degradation products \textit{in vivo}. There have been many reports of immunologic responses temporally associated with implantation of metal components,\(^2,\)\(^3\) where metals known as sensitizers (haptenic moieties in antigens) include beryllium, nickel, cobalt and, chromium.\(^3\) However, before a complete understanding of the potential adverse immunogenic consequences of Ti and Co-Cr-Mo orthopedic alloys, their respective dissolution properties, i.e. metal release kinetics and serum protein binding characteristics, must be characterized. We hypothesize that Co-Cr-Mo and Ti-alloy biomaterials may differentially release metal release into serum, with different levels of serum-metal saturation for Ti and Cr constituents, and differential metal-protein binding. These may all act to mediate any subsequent differential bioreactivity of these two implant alloys. In this investigation we determine the Cr and Ti release and protein binding kinetics associated with two primary orthopedic alloys used in total joint arthroplasty: Co-Cr-Mo alloy ASTM F-75 and Ti alloy F-136.

MATERIALS AND METHODS: Differential Cr and Ti release from Co-Cr-Mo and Ti implant alloy beads into human serum and the differential adsorption of serum protein upon the surface of these particles was measured using human serum from 5 subject volunteers, with approved consent from the internal review board \((n=5, 1\text{ male and }3\text{ females, average age 54})\) and fetal bovine serum \((\text{FBS, Sigma})\). The release of metal from an implant was induced by incubating 0.5 ml of spherical particles \((70\text{µm dia.})\) of 1) pure titanium (cpTi), 2) titanium alloy \((\text{Ti6Al4V, ASTM F-163})\) and 3) Co-Cr-Mo alloy \((\text{ASTM F-75})\) (Starterm Corp., Concord, MA) in 4 ml of serum under constant rocking motion \((\text{LabQuake, Sigma Co, MO})\) for one week. Samples of this serum were taken at 0, 0.5, 2, 8, 24, 72, and 168 hours. Residual collooidally suspended metal particles were removed from the serum samples using centrifugation at 8,800 rpm for 10 min \((\text{Serovall, DuPont})\). Metal content within serum samples was analyzed using a graphite furnace Zeeman atomic absorption spectrophotometer \((\text{GFZ-AAS, Perkin-Elmer, Norwalk, CT})\). The method detection limits were 0.03 ng/ml for Cr and 2.0 ng/ml for Ti. All collection containers and apparati were triple acid-washed with Ultrex-Norwalk, CT. The method detection limits were 0.03 ng/ml for Cr and 2.0 ng/ml for Ti. The release kinetics of Cr and Ti from cpTi, Ti-6Al-4V and F-75 were determined at a constant 

RESULTS: The release kinetics of Cr and Ti from cpTi, Ti-6Al-4V and F-75 were determined using a 10% buffered saline \((\text{PBS})\) to remove non-adherent proteins and then air-dried. Biofilm analysis. After one week of incubation time the particles in serum \((0.5 \text{ ml/sample})\) were transferred to a 50ml centrifuge tube and washed 3x with sterile phosphate buffered saline \((\text{PBS})\) to remove non-adherent proteins and then air-dried. Biofilm proteins were eluted from the Ti and Co-Cr-Mo particles using 200 µl of 2% sodium dodecyl sulfate \((\text{SDS})\) under constant rocking \((\text{LabQuake, Sigma})\) for 24 hours. The resultant eluant biofilm solutions were analyzed using a 10% polyacrylamide gel \((\text{SDS-PAGE})\). Gel densitometry was conducted using a gel scanner \((420oe, \text{PDI})\) and ScionImage image processing software for determination of total and peak protein amounts.


![Figure 1](image1.png)  
**Figure 1.**  

![Figure 2](image2.png)  
**Figure 2.**