INTRODUCTION:

Endotoxin adhering to the different particles examined in each study. Previous studies might be the result of differing levels of contaminating endotoxin reported for wear particles, we hypothesized that the discordant results in previous studies might be the result of differing levels of contaminating endotoxin adhering to the different particles examined in each study.

MATERIALS AND METHODS:

Commercially pure titanium (cpTi) particles (1-3μm, Johnson Matthey) were tested for endotoxin levels using the high sensitivity version of the spectrophotometric Limulus Amoebocyte Lysate (LAL) assay (detection limit = 0.005 EU/mL, BioWhittaker). CpTi particles were added directly to the LAL assay during the reaction period and were removed by centrifugation prior to determining the absorbance values. Efficiency of removal of the particles was confirmed by measuring color blanks in all assays. Spikes containing known amounts of endotoxin were also measured to determine whether high concentrations of cpTi particles inhibit the LAL reaction. As recommended by the manufacturer, assays of samples with recoveries of spikes less than 75% of expected were considered to be unreliable. The measured amount of endotoxin/10^6 particles was converted to EU/cm^2 using the spectrophotometric specific surface area of the cpTi particles determined by Coulter Counter analysis (Ricerca, Inc., Painesville, OH). Endotoxin was also measured on discs of cpTi or Ti-6Al-4V alloy that had been prepared by the manufacturer exactly as they process implants. That is, they were grit blasted to a 125 RA finish, cleaned, passivated, individually packaged, and gamma sterilized. Endotoxin levels were determined by adding the discs directly to the high sensitivity LAL assay as described above except that the assay volume was increased in order to completely submerge the discs.

RESULTS:

This study was designed to assess the possibility that adherent endotoxin may exist on orthopaedic wear particles and may, therefore, be responsible for many of the biological responses that have been attributed to orthopaedic wear particles other than cpTi from Johnson Matthey. However, since endotoxin is ubiquitous in the laboratory environment [4], it is likely that at least some other particle preparations also contain significant amounts of adherent endotoxin. Our results show that it is essential to measure endotoxin on the particles themselves rather than on particle-free supernatants as endotoxin is extremely adherent. It is also essential to include endotoxin spikes in the assays as the particles can inhibit the reaction. Our results also demonstrate that orthopaedic implant surfaces contain significant levels of adherent endotoxin. Thus, endotoxin may also be important in the development of aseptic loosening in patients.

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REFERENCES: