ETHANOL OR ACID WASHING DOES NOT REMOVE LIPOPOLYSACCHARIDE FROM TITANIUM OR POLYETHYLENE PARTICLES

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Background:
Particulate wear debris-induced osteolysis is a primary reason for aseptic loosening of implants. Recently, studies indicated that lipopolysaccharide (LPS or endotoxin) contamination of the particles markedly enhances their biological activity. Ragab et al. pointed out that most of the cytokines released from mouse marrow cells in response to commercially available titanium (Ti) particles were due to contamination with endotoxin. In addition, measurable amounts of endotoxin have been found on the surface of commercially available prostheses. Decontamination of LPS is therefore significant in the study of the biological effects of particles, and especially in implant manufacture and cleaning.

In the literature, many methods have been used to clean Ti or polyethylene particles, such as vigorous washing with ethanol, acetic acid, hydrochloric acid (HCl), autoclaving, or baking in an oven (Cho et al.). Although these studies found little to no LPS after the cleaning processes, the eluates or suspensions of the particles were tested using the Limulus Amoebocyte Lysate (LAL) assay. Due to the presence of particles and the small amount of LPS absorbed on the particles, this assay may not have been able to accurately determine residual amounts of LPS on the particles. Also, there is not much known about the interactions of orthopaedic-related particle debris and LPS. In our experiment, we used radiolabeled LPS to study the effect of different methods of cleaning on Ti and on high density polyethylene (PE) particles.

Materials and Methods:
Wear debris Ti particles (diameter 3.00 ± 2.62 microns) and PE particles (diameter 4.73 ± 2.11 microns) were obtained from Smith & Nephew, Inc. All particles were washed with 95% ethanol (EtOH) 5 times. Ti particles were further cleaned by heating at 160°C for 24 hours. Radioactive LPS from Salmonella typhimurium PR122 was purchased from List Biological Laboratories (Product #508). The N-acetyl-glucosamine had been labeled with 14C which was incorporated into the R core and lipid A regions of LPS. This radiolabeled LPS had a specific activity of 0.5 microcuries/mg. The LPS was dissolved in PBS at a concentration of 0.78 µg/ml. Ti and PE particles (1.34 mg) were incubated in 300 µl 14C-labeled LPS (0.78 µg/ml). For Ti particles, this was performed in a 1.5ml plastic Eppendorf tube; for PE particles, a tube with a chamber and filter was used. After vortexing for 1 minute, tubes were left at room temperature for 24 hours. The residual solution was harvested as “residue”. All particles were washed with endotoxin-free PBS twice. After that, four methods of cleaning were used, with 5 tubes for each method (300 µl/wash); they were: washing with 1 N HCl twice; washing with 95% EtOH twice; washing with 10% Triton X-100 twice; and washing with PBS twice after baking at 160°C for 24 hours (Ti only). The washes were harvested as “1st wash” and “2nd wash”. The radioactivity of the washes and particles was examined with a scintillation counter.

The results of radioactivity remaining on the particles after washing were statistically analyzed using ANOVA (one-way analysis of variance) to ascertain whether there was a difference among the groups, and post-hoc testing using Tukey test was performed to isolate differences. P<0.05 was considered significant.

Results:
Most LPS was absorbed by the particles and the tube, as residue counts were low (Figures 1 & 2). Triton X-100 was more effective in reducing LPS counts on the particles, especially the PE particles, than was EtOH or HCl washing (P<0.05). Washing with PBS after heating Ti particles to 160°C for 24 hours significantly reduced LPS counts left on the particles compared to the EtOH, HCl or Triton methods (P<0.05). There was no statistical difference between HCl washing and EtOH washing for Ti or PE particles.

Figure 1. Radioactive LPS bound to Titanium particles after washing. 600 CPM/tube was initially added to the particles.

Figure 2. Radioactive LPS bound to PE particles after washing. 600 CPM/tube was initially added to the particles.

Discussion:
From our results, LPS has high affinity for Ti and PE particles. We found that EtOH and HCl washes were not effective in removing LPS from Ti and PE particles. The same procedures have been reported in the literature to create “clean” particles. This may be a reason for the ambiguous results reported in the literature concerning the effects of “cleaning” particles on cytokine secretion by macrophages. Another factor for the variability of these methods might be chemical alteration of the carbohydrate moiety of LPS such that it is no longer specifically recognized by CD14, the macrophage toll-like receptor 4. The effectiveness of Triton X-100 indicates that detergents are more useful in decontaminating PE. For Ti, heating at high temperature is a better choice as it makes the endotoxin easier to wash off. In our experiments, both Ti and PE particles were initially incubated in a solution of LPS in PBS. Since PE particles are hydrophobic and clump together, their contact with LPS during this incubation may have been limited, even with vortexing. Thus the exposure of LPS to these two different particles may not be completely comparable in these experiments.

Reference:

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