



In vitro studies on the effect of particle size on macrophage responses to nanodiamond wear debris

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ARTICLE INFO

Article history:

Received 19 September 2011

Received in revised form 23 January 2012

Accepted 25 January 2012

Available online 2 February 2012

Keywords:

Diamond
Nanoparticles
Wear debris
Macrophage
Osteolysis

ABSTRACT

Nanostructured diamond coatings improve the smoothness and wear characteristics of the metallic component of total hip replacements and increase the longevity of these implants, but the effect of nanodiamond wear debris on macrophages needs to be determined to estimate the long-term inflammatory effects of wear debris. The objective was to investigate the effect of the size of synthetic nanodiamond particles on macrophage proliferation (BrdU incorporation), apoptosis (Annexin-V flow cytometry), metabolic activity (WST-1 assay) and inflammatory cytokine production (qPCR). RAW 264.7 macrophages were exposed to varying sizes (6, 60, 100, 250 and 500 nm) and concentrations (0, 10, 50, 100 and 200 $\mu\text{g ml}^{-1}$) of synthetic nanodiamonds. We observed that cell proliferation but not metabolic activity was decreased with nanoparticle sizes of 6–100 nm at lower concentrations (50 $\mu\text{g ml}^{-1}$), and both cell proliferation and metabolic activity were significantly reduced with nanodiamond concentrations of 200 $\mu\text{g ml}^{-1}$. Flow cytometry indicated a significant reduction in cell viability due to necrosis irrespective of particle size. Nanodiamond exposure significantly reduced gene expression of tumor necrosis factor- α , interleukin-1 β , chemokine Ccl2 and platelet-derived growth factor compared to serum-only controls or titanium oxide (anatase 8 nm) nanoparticles, with variable effects on chemokine Cxcl2 and vascular endothelial growth factor. In general, our study demonstrates a size and concentration dependence of macrophage responses in vitro to nanodiamond particles as possible wear debris from diamond-coated orthopedic joint implants.

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1. Introduction

Data from the American Academy of Orthopedic Surgeons show that more than 418,000 total and partial knee replacements and 328,000 total and partial hip replacements are performed in the US each year, and the number of total knee replacements and total hip replacements performed in the US is expected to leap by 673% and 174%, respectively, by the year 2030 [1]. Wear of articulating surfaces involving cobalt–chromium–molybdenum (CoCrMo) alloy against polyethylene (components in the majority of hip and knee implants) has been cited as a dominant factor limiting the long-term success of the implants [2,3]. Wearing and the resultant generation of wear debris particles can lead to mechanical instability, decreased joint mobility, increased pain, deleterious biological responses, osteolysis, and ultimately component loosening and implant failure [2–4]. Large debris pieces are normally sequestered by fibrous tissue, while small debris is phagocytosed by macrophages and monocytes, which may release cytokines that result

in inflammation. The inflammation cascade also leads to the recruitment of activated osteoclasts at the bone–implant interface and to bone resorption around the implant. One solution for this problem of osteolysis caused by wear debris is to coat harder materials, such as diamond, on the articulating surfaces to improve wear resistance and to reduce the number and size of debris particles generated.

Studies have shown that less wear occurs with metal-on-metal hip prostheses, up to 100 times less than that of polyethylene-on-metal, and CoCrMo metal articulation results in smaller debris particles than metal-on-polyethylene articulation [5–7]. The CoCrMo debris from a metal-on-metal articulation were shown to be in the range of 6–744 nm, with an average size of 42 nm [6], whereas polyethylene particles ranged from 0.1 to 5 μm (100–5000 nm) [8]. As the metal wear-particles are relatively smaller than polyethylene particles, they may be less prone to inflammation and osteolytic reaction. A study investigating bone and tissue reactions to metal debris in several metal-on-metal components revealed that there were fewer macrophages and giant cells than typically seen in tissues around metal-on-polyethylene joints [2]. Unfortunately, there are concerns and debate associated with metal sensitivity

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