INTRODUCTION: Particulate debris generated from continuous prosthetic wear can lead to aseptic loosening of the implant[1]. Titanium wear particles have been documented in periprosthetic bone and bone marrow (including remote sites such as the iliac crest), suggesting direct and chronic exposure of marrow cells to orthopaedic wear debris[2]. In this study, we investigate the effects of submicron wear particles on human mesenchymal stem cell (hMSC) differentiation into a functional osteoblast phenotype by evaluating gene expression of osteoblast markers (alkaline phosphatase, osteocalcin, and bone sialoprotein) and production of bone matrix proteins (collagen type I and bone sialoprotein) in the presence of submicron titanium and zirconia particles.

MATERIALS AND METHODS: Particle Preparation: Commercially pure titanium (Sigma) and zirconium (IV) oxide (Aldrich) particles (0.5 μm) were treated with 25% nitric acid, washed with sterile phosphate buffered saline (PBS), and re-suspended in DMEM/10% fetal bovine serum (FBS). Limulus assay of particle suspensions excluded endotoxin levels exceeding 0.06 EU/ml.

MSCs isolation and culture: hMSCs were isolated from the bone marrow derived from the femoral heads of patients undergoing total hip arthroplasty (THA) for primary osteoarthritis. Tissues from patients with avascular necrosis, congenital malformation, neoplasia, or viral infection were excluded (this project has TJU-IRB approval and complies with all requirements for patient consent). Cells were re-suspended in DMEM/F12 supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin, seeded at 3x10^6 cells/ml/well in T150 tissue culture flasks, and allowed to adhere for 48 hours. Cells were supplied fresh medium every 72 hours and expanded for 2-3 weeks (until 80% confluence) prior to use.

Cell treatment: hMSCs were cultured in αMEM/10% FBS containing ZrO_2 and cpTi particles (approximately 5000 particles/cell) or vehicle for 24 hours. Following particle loading, cells were cultured in fresh medium supplemented with osteogenic factors (50 mg/ml L-ascorbate-2-phosphate, 10^-7 M β-glycerol phosphate, and 10^-4 M 1α, 25-(OH)2D3) every three days and allowed to differentiate for 12 days. As a negative control, non-particle loaded cells were cultured in α-MEM/10% FBS without osteogenic agents.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR): Total RNA was isolated with TRIzol reagent (GibcoBRL) from cell cultures and analyzed by RT-PCR for RNA expression. Gene specific primers for glyceraldehyde phosphate dehydrogenase (GAPDH), alkaline phosphatase (AP), collagen type I (Col I), osteocalcin (OC), and human bone sialoprotein (BSP) were used for RT-PCR. RT-PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium-bromide staining and densitometrically analyzed relative to GAPDH.

Western analysis: Following cell lysis with RIPA buffer, total protein was isolated and equal amounts of protein extracts were separated by SDS-PAGE electrophoresis and transferred to nitrocellulose membrane (Amersham) for Western assay. Polyclonal rabbit antiserum (generously provided by Dr. Larry Fisher, NIH) to human BSP (LF-100) and human collagen type I (α chain) (LF-39) were used to detect production of BSP and Col I. Western blots were developed using chemiluminescent detection reagents and exposed to ECL Hyperfilm (Amersham) accordingly.

RESULTS: Following 12 days of treatment with osteogenic (OS) medium, marrow-derived hMSCs isolated from the femoral head and expanded in vitro are capable of differentiating into an osteoblastic cell type, exhibiting increased expression of AP, OC, and BSP (Fig. 1). Col I was constitutively expressed by both treated and control cells. Western analysis of OS-treated cells revealed a significant increase in Col I and BSP compared to control (Fig. 2). During this differentiation process, exposure to submicron cpTi particles suppressed the ability of hMSCs to differentiate into a functional osteoblastic phenotype, resulting in decreased BSP gene expression as well as reduced BSP and Col I protein production. mRNA levels of AP, Col I, or OC were not significantly affected following exposure to cpTi.

REFERENCES:

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Fig. 1. RT-PCR: Following treatment with osteogenic medium (OS), hMSCs isolated from the femoral head exhibited increased expression of AP, OC, and BSP. Exposure to cpTi (OS+cpTi) particles resulted in a decrease in BSP gene expression, while exposure to ZrO_2 particles (OS+ZrO_2) of similar size did not have a significant effect.

Fig. 2. Western analysis: hMSCs treated with osteogenic factors (OS) exhibited increased Col I and BSP production. In the presence of cpTi particles, Col I and BSP synthesis was reduced. Exposure to ZrO_2 particles did not significantly affect production of either protein.

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