Published in final edited form as:

Arthroscopy. 2013 September ; 29(9): 1559–1565. doi:10.1016/j.arthro.2013.05.030.

Arthroscopic surgical tools: A source of metal particles and possible joint damage

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Abstract

Purpose—Our goals were (1) to characterize metal micro-particles created by standard arthroscopic instruments, and (2) to examine the in-vitro cellular responses induced by those particles, including possible synergistic effects with local anesthetic.

Methods—We applied standard surgical tools to 16 foam bone blocks immersed in saline (plus 3 non-instrumented controls). Eight specimens had four minutes of exposure to a 4.0 mm full radius shaver rotating forward at 6,000 RPM. In the other blocks, four holes were created with a 3.0 mm drill via a sleeve. Particles were isolated onto silicon wafers by density gradient ultracentrifugation, and SEM analyzed a minimum of 1000 particles per wafer. Metal particles were then isolated and purified. Aliquots of sterilized micro-particles were applied to cultured bovine chondrocytes (+/- local anesthetic) and to cultured human or bovine synoviocytes. Chondrocyte viability was assessed with live/dead cell assay by flow cytometry. Synoviocyte responses were assessed with qPCR.

Results—Stainless steel or aluminum particles were found in each sample (same composition as surgical instruments). Average particle size was 1 to 2 μm (range 50 nm to 20 μm). Chondrocyte exposure (1 hour) to metal debris induced a small but statistically significant increase in cell death, without any synergistic effect of local anesthetic. Proinflammatory chemokines were consistently upregulated in both human and bovine synoviocytes exposed to metallic micro-particles for 3, 24, and 48 hours.

Conclusions—The current study demonstrates that metallic microdebris is liberated by common arthroscopic instruments, at scales much smaller than previously recognized. These particles are bioactive as demonstrated by the in-vitro synoviocyte responses initiated by metallic micro-particles.

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Clinical Relevance—Our findings suggest that metallic micro-particles could induce intra-articular damage via a synovocyte-mediated cytokine response if their concentrations reach clinically significant levels.

Introduction

Different sized metal particulates are generated in different surgical settings. The majority of these particulates are very small (at the nanometer scale). Metallic artifact has been commonly seen on magnetic resonance imaging (MRI) following arthroscopic surgery, especially following shoulder arthroscopy, while X-rays following the same procedures fail to show any metal artifact. This occurs because this metallic artifact is too small to appear on X-rays. The presence of such metal debris has been seen and documented in the radiology literature because it can create significant artifacts such as signal loss, failure of fat suppression, geometric distortion, and resonant frequency changes.¹

Metallic particle generation has also been described in total joint replacement literature. Studies have shown that metallic wear particulates are generated by prosthetic joints in the range of .02-5 microns. Such particles are phagocytized by periarticular cells, inducing an inflammatory response that results in osteolysis and aseptic joint loosening.²⁻⁴

Chondrolysis is characterized by the rapid deterioration of articular cartilage, but the pathoetiology of chondrolysis remains unclear. Various theoretical possibilities have been proposed, including post operative intra-articular infusion of local anesthetics via pain pumps (mainly with bupivacaine and lidocaine with epinephrine), thermal insult following the use of radiofrequency devices or lasers, and exposure to toxic substances such as Gentian Violet dye or methymethacrylate cement.^{5,6} However, our current understanding of chondrolysis suggests that there are probably other fundamental contributing factors, yet to be elucidated. Scheffel and co-workers found a correlation between an increased rate of chondrolysis and an increased number of suture anchors used for the arthroscopic procedure, but the pathogenic mechanism of that association was unexplained.⁶

Our goals were (1) to characterize metal micro-particles created by standard arthroscopic instruments, and (2) to examine the in-vitro cellular responses induced by those particles, including possible synergistic effects with local anesthetic. Our hypothesis was that metallic micro-debris could induce pathogenic responses at the cellular level.

Methods

Specimen Preparation

A total of nineteen foam specimens (Sawbones, Inc., Vashon, WA) having density close to the cortical bone were cut from one sample of foam using a machine shop band saw, to create blocks of identical size (2×2×2 cm). After cutting, each block was rinsed with alcohol and cleaned by sonication for 10 minutes to remove artifacts from the saw blade.

Simulated arthroscopic procedures were performed with the foam blocks immersed in 10 cc of room temperature normal saline by a single experienced arthroscopic surgeon, within labeled plastic specimen containers that were sized to firmly stabilize the foam blocks.

The tests performed were as follows:

- A. Eight specimens were subjected to four minutes of exposure to a 4.0 mm full radius arthroscopy shaver (Stryker Inc., Mahawah, NJ) rotating in the forward direction at 6,000 RPM. At the completion of shaving, the shaver hand piece and blade were

rinsed with 15 cc saline into the saline bath. A fresh shaver was used for each foam bone specimen.

- I. SoffB (Shaver off Bone): Four specimens involved shaver rotation, without foam debridement (non-cutting edge of shaver toward bone).
  - II. SonB (Shaver on Bone): Four specimens involved shaver rotation, with cutter contact to the foam, with sufficient pressure to debride the surface of the foam (cutting edge toward bone).
- B. Eight specimens were subjected to four drill holes (in each specimen), which were created with a metal drill within a metal drill sleeve (arthroscopic instrumentation for the 3.0 mm BiosutureTak, Arthrex, Naples, FL). At the completion of drilling, the sleeve was cleared with a BiosutureTak implant and then rinsed with 15 cc saline into the saline bath. A fresh drill was used for each foam bone specimen. One drill sleeve was used for all the specimens in condition I, and a second fresh sleeve was used for all the specimens in condition II, below.
- I. DnB (Drill no Bend): Four involved drill rotation in the sleeve, to a constant depth, without bending of the drill (no bend condition).
  - II. DwB (Drill with Bend): Four involved drilling into foam, to a constant depth, with slight bending of the drill within the drill sleeve (bend condition). This condition was intended to mimic the clinical situation whereby the drill is not exactly co-linear with the drill sleeve, which could hypothetically increase frictional forces between the two metal surfaces.
- C. Three of the specimens were immersed in the saline bath, and the blocks and fluid bath were used as controls, without any additional manipulations.

Isolation of Particles for Scanning Electron Microscopy (SEM) Analysis

Metal particulates were purified, quantified and characterized, and isolated for biological testing, adapting the protocol developed by Billi and co-workers.⁸ All sample preparation was performed in certified laminar flow clean hoods. All applied water was purified by deionization and filtered through a 0.02 μm filter. Sodium azide (0.04% NaN_3) was added to the saline immersed test samples to retard biomass growth. Saline test samples were sonicated for 1 minute 4 times at 100% amplitude, 0.8 cycle, in closed polypropylene centrifuge tubes using a Vial-tweeter/UIS250L non-invasive sonicator (Hielscher, Ringwood, NJ) to homogenize the particle distribution. Aliquots of saline were then centrifuged (Beckmann Optima XL80) through a density gradient, onto a Si wafer surface previously treated with an organic adhesive following a previously defined isolation protocol.⁸

Morphological and Chemical Characterization of Particles

The silicon wafers obtained after ultracentrifugation, were iridium sputter coated (IBSe, Southbay Inc., San Clemente, CA). A Field-Emission Scanning Electron Microscope (FE-SEM, Supra 40VP, Zeiss, Peabody, MA) was used at an acceleration voltage of 15kV to analyze each wafer at three different magnifications (FOV of 28 μm^2 , 453 μm^2 , 7242 μm^2). Two areas were analyzed for each magnification. A minimum of 1000 particles per wafer were characterized for morphology, unless specified. Four different morphological descriptors: maximum caliper length (dmax), maximum caliper length perpendicular to dmax (dmin), equivalent circular diameter (ECD), and aspect ratio, dmax/dmin (AR) were recorded for each analyzed particle.

Energy-dispersive spectroscopy (EDS) analysis (Thermo Ultradry feature sizing system) was performed simultaneously to morphological analysis to identify the chemical composition of the particles. Particles were broken down into the following groups: Aluminum, Stainless Steel, and Other. Stainless steel was defined as particles containing iron and/or chromium. The EDS spectra of some particles contained both aluminum and stainless steel. In these cases, any particle whose mass percentage of aluminum was greater than the combination of iron and chromium was defined as aluminum. If the particle contained more iron, chromium or a combination of the two, the particle was defined as stainless steel. EDS was successfully applied for chemical characterization of particles as small as 12 nm. Particles smaller than 10 nm could not be characterized since the EDS signal was blended into the background spectrum.

Cell Culture

Normal human synoviocytes were obtained from a two-year old donor during a previous study.⁹ Normal bovine articular chondrocytes and synoviocytes were obtained from the stifle joints of three-week-old calves provided by an abattoir. The cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Carlsbad, California) in a humidified incubator with 5% CO₂ at 37°C.

Cell Viability Assays

To assess the effects of different dosages of particulate on chondrocytes, approximately 0.3 million bovine articular chondrocytes were suspended in phosphate-buffered saline (PBS) and placed into 12×75-mm plastic non-permeable test tubes. The cells were centrifuged at 1,000 rounds per minute for five minutes. After discarding the supernatant, 0.3 mL of PBS or particle solution was added to each tube. The cells were re-suspended by gently pipetting three times. Then, bupivacaine (Hospira, Inc, Lake Forest, Illinois) was added into the cell suspension to a final concentration of 0.125%. The control and treatment groups are shown in Table 1. The cells were kept in suspension by means of gentle vortexing once every five minutes and were incubated at 37°C for one hour. Finally, the cells were washed three times with PBS and processed for flow cytometry analysis. The experiments were performed 2 times independently.

Flow Cytometry Analysis of Cell Viability

The treated chondrocytes were stained with a solution containing 0.8-μM calcein AM (acetoxymethyl ester) and 0.8-μM ethidium homodimer-1 in PBS for twenty minutes in the dark at room temperature. The reagents were obtained from the LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, Eugene, Oregon). Live cells emit green fluorescence while dead cells emit red fluorescence. Percentages of live and dead cells were obtained by counting 10,000 cells per sample using a Becton-Dickinson Life Science Research II (BD LSRII) flow cytometry analyzer (Becton, Dickinson and Company, San Jose, California).

Quantitative Polymerase Chain Reaction (PCR) Analyses

About 0.6 million human or bovine synoviocytes were plated in 60-mm dishes with Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA) and incubated in a humidified incubator with 5% CO₂ at 37°C for overnight. Then, metal particles were added to each dish in 6:1 ratio (i.e., 6 particles per cell based on our cell viability studies with the particles) for 3, 24, and 48 hours. RNAs were isolated from the cells using an RNeasy kit (QIAGEN, Valencia, CA); reverse transcription was performed using Superscript™ First-Strand Synthesis System (Invitrogen, Carlsbad, CA); realtime PCR was performed on the iQ5 PCR instrument with

SYBR Green reagents (Bio-Rad Laboratories, Hercules, CA). The mRNA levels were first normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and then the relative mRNA fold change was calculated as $2^{\Delta\Delta C_t}$ compared to the basal levels. The treatment groups were normalized to a PBS treated control group. The experiments were independently performed 3 times with bovine synoviocytes and 2 times with human synoviocytes.

Statistical Analysis

The means and standard deviations (error bars) of a representative experiment were presented for each study. Comparisons between the control and treatment groups were analyzed by use of the 2-tailed Student *t* test. We did not do multiple comparisons using the Student *t*-test. Each *t*-test was for comparisons between two groups, that is, a non-treated control group versus a group treated at a time point. The level of significance was set at $P < 0.05$.

Results

Micro sized stainless steel (primarily iron and chrome) and aluminum particulates were generated from both the shaver and metal drill bit/drill sleeve. Specimen SoffB1 was an outlier, with more than 6 times the particulate debris compared to the other samples. The etiology of this discrepancy was unclear.

The SonB condition (samples 1-4) generated 7.8% more particulate than the SoffB condition (samples 2-4). The chemical makeup of the generated particulates varied between the SonB and the SoffB conditions. The SonB condition generated more aluminum than stainless steel. The SoffB condition generated more stainless steel than aluminum. The majority of particulates generated were between 0.05-1 micron in size (Figure 1).

Both the Drill-with-Bend and the Drill-no-Bend conditions also generated stainless steel and aluminum particulates. The Drill-with-Bend condition generated significantly more stainless steel particulates than the Drill-no-Bend condition. The drill no-bend condition generated more aluminum particulates than the drill with bend condition. The majority of particulates generated were between 0.05-1 micron in size.

Control samples also contained stainless steel and aluminum particulates but close analysis revealed distinct differences between the particulates in the control and the particulates in the other four conditions. Larger particulates were present in the samples but not in the controls, indicating that such particulates must have been generated from either the drill or shaver.

As shown in Figure 2, we found that the control group had a cell death rate of approximately 5%. Metallic-micro particles, with or without bupivacaine, increased the cell death rate to about 6% to 9%. However, only the bupivacaine only, bupivacaine plus 2×DwB particles, and 2×SoB particles groups showed that the increase of cell death was of statistical significance. Synergistic effects of particulate and local anesthetic on chondrocyte viability were not observed.

As shown in Figure 3, we found that the four types of metal particulates acted on bovine synoviocytes and induced expression of chemokines (CXCL1, CCL2, and IL-8), cytokines (IL-6 and TNF α), and matrix-degrading enzymes (MMP-9, MMP13, ADAMTS-4, and ADAMTS-5), though the magnitudes of induction varied among the different particles over the time course of 3 hours, 24 hours, and 48 hours.

Similarly, we found that the four types of metal particulates induced expression of the chemokines, cytokines, and matrix-degrading enzymes in human synoviocytes (Figure 4).

Discussion

Small metal particulate can be observed in joints both during and after arthroscopic surgery, sometimes by direct arthroscopic visualization and at other times indirectly by the imaging artifact induced on post-operative MRI. The potential implications of such particulate are for the most part unexamined in the arthroscopic literature. Maletis and co-workers studied 10 rabbit knees that were subjected to an intra-articular injection of metal debris, with particles ranging in size from 30 – 150 microns (much larger than the particles observed in the current study). No significant mechanically-abrasive cartilage degeneration was noted but it is possible that the particulate used in that study was too large to cause any significant biological joint response.⁷ Particle size has not been defined or well-classified in prior arthroscopy literature. There is very little prior work in this area. In prior animal studies⁷, the particle sizes studied were an order of magnitude larger than what we observed in the current study. Larger particles cannot be ingested into cells and therefore would not induce the same kind of cellular reaction as the tiny microparticles observed in the current study. On the other hand, larger particles could create an abrasive reaction, which we do not think would be a significant issue with microparticles.

The present study was not designed to analyze potential abrasive effects of much smaller microparticles upon chondrocytes or synovium. However, we predict that direct mechanical damage from metallic microdebris would be relatively limited, based upon the observations of Maletis and co-workers.⁷

Stainless steel and aluminum nano particles in a size range from 50 nm to 20 μ m were generated by both the drills and shavers following manipulations performed by an experienced surgeon in a manor mimicking typical arthroscopic manipulations. Analysis of the shaver revealed that the shaver tip and sleeve were comprised of stainless steel. Furthermore, the sleeve was also chromium plated. An alumina anti-seizing paste was also identified between the shaver and shaver sleeve. Analysis of the drill revealed that the drill shaft and sleeve were made of a stainless steel. The drill handle was made of anodized aluminum. The handle acted as a bearing surface for the drill. The drill cuff rubbed against the aluminum handle generating aluminum particles. Circular scratching of the handle was severe enough to wear off the purple anodized aluminum.

The control samples were also found to contain some stainless steel and aluminum particulate. These particles were smaller in size and had a distinct morphology, and were distinguishable from the metal particles that were generated by the arthroscopic instruments. This small amount of background contaminant may have been created by the saw blade that was used to cut the foam bone specimens.

Isolated particles were used in the current in-vitro tests to evaluate the effects on chondrocyte viability. A small increase in cell death was observed when the metal particles were placed in contact with cultured chondrocytes that had been removed from the cartilage matrix. We hypothesize that metal microparticles may not cause dramatic and direct cartilage injury in-vivo because chondrocytes are protected by the matrix. We did not observe overt synergistic effects between metal particles and exposure to bupivacaine upon cultured chondrocytes. Metallic particulates were able to induce expression of chemokines (CXCL1, CCL2, and IL-8) and cytokines (IL-6 and TNF α) in bovine and human synoviocytes. These chemokines and cytokines are known to be able to recruit inflammatory cells and/or inhibit matrix synthesis by the chondrocytes. We speculate that these

inflammatory factors may cause inflammatory responses in the joint. Further, induction of the matrix-degrading enzymes such as MMP9, MMP13, ADAMTS-4, and ADAMTS-5 may directly cause degradation of articular cartilage. Therefore, the metallic particles are likely to cause a cascade of inflammatory responses, leading to damage of the cartilage and synovium. Future studies should focus on quantification of particle generation in-vivo, in addition to further exploration of the cellular responses that may be associated with exposure to metallic microdebris in the clinical setting.

We believe that there are some important basic science observations from the field of particle pathobiology associated with failed total joint arthroplasty, that are relevant to the current study. That research indicates that the cell response to particulate debris is a function of the size, composition, and dose of the particles.⁸⁻¹⁰ Other more subtle factors that may influence particulate biocompatibility include the aspect ratio, surface roughness of the particles, and the composition of absorbed proteins, which are currently less completely understood. Metal-on-metal wear in artificial joints produces mostly nanometer-size particles that can corrode and become detectable in the blood and urine as metal ions.¹⁰ The processes involved in the formation of metal wear products and potentially antigenic haptens are poorly understood. The various corrosion products vary in size, stability, and solubility, and consequently in their bioavailability and inflammatory and immunogenic potentials. Particle aggregates and larger particles interact with the phagocytic cells, causing release of potentially osteolytic cytokines. Wear products from metal-on-metal hips are associated with B and T lymphocytes as well as plasma cells. There are occasional reports of extensive necrosis, enlarged bursae, and groin masses occurring around metal-on-metal joints, but it is unclear whether these are caused by elevated levels of wear or corrosion products or by patient hypersensitivity. We did not investigate the potential adverse effects of corrosion of metal microparticles in the current study.

Limitations

Metallic particulates have been observed visually by arthroscopists at surgery, on post-operative radiographs after anterior cruciate ligament reconstruction¹¹ and by MRI imaging following arthroscopic surgery. However, prior research has generally evaluated effects of relatively large metal particles, with a focus upon the associated risk of direct mechanical abrasion of articular cartilage. Based upon the current experimental data, we are not sure if the particles of the size and chemical composition observed in the current study are present in large quantities within human joints after arthroscopic surgery. Furthermore, even if such particles are liberated by arthroscopic instruments, the current in-vitro study does not allow us to estimate the concentration, anatomic distribution, or biologic duration of action of such particles in the clinical situation. Another limitation of the current study was that we did not examine the effects of microparticles upon chondrocytes (+/-local anesthetic) in the simultaneous presence of synoviocytes. Such complex interactions will probably require in-vivo studies in animal joints.

Conclusion

The current study demonstrates that metallic microdebris is liberated by common arthroscopic instruments, at scales much smaller than previously recognized. These particles are bioactive as demonstrated by the in-vitro synoviocyte responses initiated by metallic micro-particles.

Acknowledgments

Z.Y. received a research grant from the National Institute of General Medical Sciences (8P20GM103518-09) of the National Institutes of Health.

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Figure 1.

Micro-particles generated by arthroscopic instrumentation of foam bone.

Figure 1A: SoffB = Shaver off bone; SonB = Shaver on bone; DnB = Drill no bend; DwB = Drill with bend. [The first group excludes SoffB specimen #1, an outlier, so N=3 for that group. N=3 for the control specimens. N=4 for all other data.]

Figure 1B: SEM of a control sample (magnification marker = 1 micron). Figure 1C: SEM of a drill with bend sample (magnification marker = 1 micron).

Figure 2.

Effects of metallic micro-particles on chondrocyte viability. Bovine chondrocytes were suspended in PBS in the test tubes and incubated with micro-particles, with or without 0.125% bupivacaine, at 37°C for 1 hour; after staining with the live/dead reagents, the cells were analyzed using flow cytometry. The bars represent the means of cell death rates after treatment. Error bars represent the standard deviations. 2× means a ratio of 2 particles per cell and 6× means a ratio of 6 particles per cell. DwB, Drill with Bend; SoB, Shaver on Bone. [*p < 0.05; **p < 0.01, compared to the PBS control group.]

Figure 3.

Effects of metallic micro-particles on gene expression in bovine synoviocytes:

Figure 3A. Effects of Drill-with-Bend micro-particles; * $p < 0.05$, compared to the control group.

Figure 3B. Effects of Drill-no-Bend micro-particles; ** $p < 0.01$; *** $p < 0.001$, compared to the control group.

Figure 3C. Effects of Shaver-off-Bone micro-particles; ** $p < 0.01$; *** $p < 0.001$, compared to the control group.

Figure 3D. Effects of Shaver-on-Bone micro-particles; ** $p < 0.01$; *** $p < 0.001$, compared to the control group.

Figure 4.

Effects of metallic micro-particles on gene expression in human synoviocytes:

Figure 4A. Effects of Drill-with-Bend micro-particles; * $p < 0.05$; ** $p < 0.01$, compared to the control group.

Figure 4B. Effects of Shaver-on-Bone micro-particles; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared to the control group.

Table 1
Experimental Groups in Cell Viability Assays

Groups	Type of Particles	Particles per Cell	Concentration of Bupivacaine
PBS		0	0
0.125% bupivacaine		0	0.125%
2×DwB	DwB	2	0
6×DwB	DwB	6	0
2×DwB+0.125%bupivacaine	DwB	2	0.125%
6×DwB+0.125%bupivacaine	DwB	6	0.125%
2× SonB	SonB	2	0
6×SonB	SonB	6	0
2×SonB+0.125%bupivacaine	SonB	2	0.125%
6×SonB+0.125%bupivacaine	SonB	6	0.125%

Notes: DwB used particles that were generated by the Drill with Bend condition. SonB used particles that were generated by the Shaver on Bone condition. 2× indicates 2 particles per cell; 6× indicates 6 particles per cell.