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In Vitro Assessment of Serum-Saline Ratios for Fluid Simulator Testing of Highly Modular Spinal Implants With Articulating Surfaces

Nadim Hallab, PhD,[a] Ashutosh Khandha,[b] George Malcolmson,[b] and JP Timm [b]

ABSTRACT

Background

The increasing complexity of articulating spinal implants prohibits the use of serum-supplemented simulator fluid testing because multicomponent interfaces retain residual protein and preclude gravimetric measurement. Our original hypothesis was that simulator testing of a posterior dynamic stabilization implant that has metal-on-metal articulating bearings will not produce dramatically different wear debris when tested using pure saline versus testing in saline supplemented with 20% serum.

Methods

This hypothesis was tested using simulator testing of 12 dynamic stabilization spinal implants, 6 in 100% saline and 6 in 20%-serum saline. Gravimetric and particle analysis were performed after every million cycles up to 10 million cycles, with flexion of 11.3°/extension of 5.6° coupled with axial rotation of ± 4°.

Results

The mean gravimetric weight loss was approximately 200 mg over 10 million cycles for the implants tested in 100% saline, while the mean weight loss for those tested in 20%-serum saline was below the method detection limits (< 10 mg over 10 million cycles). For the 100%-saline and 20%-serum simulator fluids, the average particle size over the course of 0 to 10 million cycles remained relatively constant at 0.2 µm-dia (saline) and 3.2 µm-dia (20%-serum saline). Testing in 100% saline generated > 1000-fold more particles, compared to testing in 20% serum-supplemented saline. Energy-dispersive X-ray (EDAX) analyses of particles demonstrated that the 100% saline debris was composed of Co-Cr-P-O (Cr-Co metal oxides), and for the 20%-serum saline debris only bulk metal Co-Cr was detected.

Conclusion

Our initial hypothesis was not supported. There were significant differences in gravimetric wear, average size, and type of wear debris that were mechanistically attributable to the type of simulator fluid used. The over-protective effect of serum proteins appears to underscore the importance of using both saline and serum when establishing upper and lower bounds of predictive implant debris generation modeling, where saline represents a worst-case scenario and as little as 20% serum masks all weight loss completely in highly modular articulating implants.

Clinical Relevance

Clinical Relevance = 5 (Oxford Centre for Evidence-based Medicine Levels of Evidence). Study findings are limited to a greater understanding of the science associated with predictive wear testing of articulating spinal implants.

Key Words: Wear, spine, implant, saline, serum, debris, metal. ***SAS Journal.*** December 2008;2:171–183. DOI: SASJ-2008-0013-RR

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INTRODUCTION

The use of spinal implants has been steadily increasing over the last 20 years, with the number of cervical and lumber fusions in the US increasing 111% to roughly 105 fusions per 100,000 people or approximately 305,000 fusions per year, from 1993 to 2003.[1] This represents a large market force driving the development of new types of spinal implants such as total disc arthroplasties (TDA) or intervertebral disc replacements (IDR) and new biologic treatment modalities. Ultimately, new technologies, such as intervertebral disc replacement, seek to replace spine fusion by both eliminating pain and preserving

spinal mobility (flexion-extension, lateral-bending, and rotation). Central to the assessment of device safety and efficacy is simulator testing prior to clinical evaluation. The utility of simulator analysis is generally twofold where (1) the amount of implant wear and degradation is assessed through the measurement of implant weight loss (gravimetric loss) and physical examination, and (2) the type of wear and corrosion debris is characterized to assess potential bioreactivity. Simulator testing for articulating total disc implants or dynamic stabilization devices is generally conducted up to 10 million cycles under loading and motion appropriate to the implant in question under the guidelines of ASTM F2423, ASTM F1717, and 2008 Investigational Device Exemption guidelines of the US Food and Drug Administration (FDA).

Understanding the gravimetric weight loss and the type of debris resulting from in vitro simulator testing is critical in predicting how an implant will perform in vivo and how much debris should be used in an animal model, prior to clinical study.

Traditional hip and knee arthroplasties enjoy a relatively high degree of standardization for predictive wear testing over the last 35 years, yet to date there remains an unclear relationship between simulator fluid composition and the effect on wear rate of different material couples in arthroplasty testing (see Table 1).[2-5] Generally, motion-sparing spine implants lack a lengthy history of empirical testing, while simultaneously posing novel and unique requirements that complicate the prediction of wear. One of these novel requirements is the increasing number of connected components and thereby the increasing complexity of articulating spinal implants when compared with traditional total hip and knee implants. Geometrical considerations of some of these designs increasingly preclude the use of serum protein-supplemented simulator fluid testing. The reason for this is that protein in serum tends to penetrate into the crevices or compartments of multicomponent devices (including protective sheaths) and then either adsorbs onto material surface or cannot be practically cleaned during weight loss measurement, thus preventing accurate gravimetric measurement of weight loss during the course of simulator testing. This complication is due to the impossibility of removing proteins from all interfaces without damaging the implants during testing.

The use of saline fluid without serum is one alternative, although without the protective effects of serum as a lubricant, articulating devices generally produce more wear under "all saline" conditions.[6] However, with little clinical data to compare with in vitro testing it remains unknown how the accurate prediction of wear debris from motion-preserving spinal implants depends on using appropriate simulator fluids for given articulating surfaces and implant constructs.

Table 1. Variety of Testing Conditions Used in Hip and Disc Simulator Studies and Differing Amounts of Wear[2-5]

Implant	Lubricant	Additive	Wear (mm³/10⁶ cycles)
Meta-Metal Hip[2] (CoCr)	70 mg/ml Bovine Serum, 90%	20mM EDTA 0.1% NaN₃	0.119 steady state
Meta-Metal Hip[2] (CoCr)	40 mg/ml Alpha Calf Serum, 50%	20mM EDTA 0.1% NaN₃	0.977 steady state
Metal-PE Hip[3] (Co-Cr-PE)	65 mg/ml Bovine serum, 4-23% 40 ml volume	20mM EDTA	55.6 average
Metal-PE Hip[3] (Co-Cr-PE)	65 mg/ml Bovine serum, 4-23% 160 ml volume	20mM EDTA	88.4 average
Metal-Metal Hip[5] (CoCr w/ varying Carbon content)	25% Calf serum	0.1% NaN₃	0.023-0.322 steady state
Metal-Metal Disc[4]	150 ml Bovine Serum (100%)	0.1% NaN₃	0.093 average

Our initial hypothesis was that simulator testing of a posterior dynamic stabilization implant that has metal-on-metal articulating bearings will not produce dramatically different wear debris when tested using pure saline versus testing in saline supplemented with 20% serum. To test this hypothesis we examined the type and amount of wear debris generated from a dynamic stabilization spinal implant using in vitro simulator testing in saline-only and in 20% serum-supplemented-saline simulator fluid over the course of 10 million cycles.
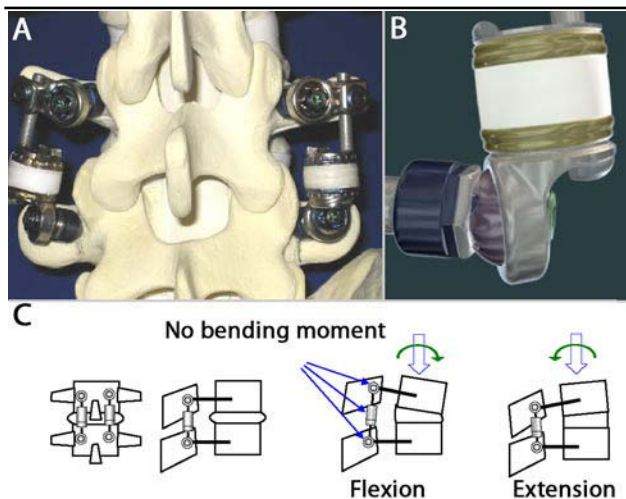
METHODS

Implant Components
The dynamic stabilization spinal implant tested is comprised of 2 titanium alloy screws with cobalt alloy ball and socket end connectors that are fixed to a cobalt alloy rod with a cobalt alloy spring housed in a polytetrafluoroethylene (PTFE) accordion flexible sheath (Figure 1). Dynamic stabilization devices were tested in flexion-extension coupled with axial rotation.

Mechanical Testing
Implant testing was conducted using simulator testing of 12 implants, 6 in pure saline (phosphate buffered saline) and 6 in 20%-serum saline solutions (20 mM EDTA + 0.2% sodium azide to prevent bacterial growth). Analysis was performed after every million cycles up to 10 million cycles. Each cyclic displacement used 11.3° of flexion and 5.6° of extension (representing a total of 17° in flexion-extension), combined with axial rotation of ± 4° to represent a worst-case loading environment for this particular device (ie, displacement control, no lateral bending, and 0 preload). Due to inherent design characteristics of the dynamic stabilization device (with multiple metal-on-metal articulating junctions, see Figure 1), larger than

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Figure 1.

(A) The dynamic stabilization spinal implant used for testing in this study is shown. Two titanium alloy screws fix the implant to the spine. (B) Two cobalt alloy ball and socket end connectors are fixed to a cobalt alloy rod imposed with a cobalt alloy spring housed in a PTFE accordion flexible sheath. (C) This design is meant to (1) provide maximum stability with minimal impact on overall Range of Motion, (2) enable a non-linear load-displacement curve with higher stiffness around neutral posture, and (3) allow for pedicle screw attachment (a well-established surgical technique).

anatomical motions were required to produce the largest reactionary force across the articulating surfaces for worst-case wear debris generation, combined with the greatest degree of relative motion between the articulating surfaces at those high loads. This reactionary force was achieved by coupling flexion-extension motion with axial rotation (without lateral bending). These displacement parameters for cyclic testing were derived from previous reports of clinical biomechanics of the spine[7] and 3D movement of the lumbar spine.[8]

These displacements were conducted at 37°C in (1) saline (PBS) with the implant polymeric PTFE sheath covering a spring assembly (2 Hz) and (2) 20% fetal bovine serum without the polymeric sheath (5 Hz). Mechanical testing was conducted following the ASTM F2423 standard guide for functional, kinematic and wear assessment of total disc prostheses and ASTM F1717 standard test methods for spinal implant constructs in a vertebrectomy model. There was no statistical increase in the temperature measured in the testing medium (< 2°C) over 10 million cycles, indicating a lack of excessive heat buildup in the devices. However, differences between the saline and serum-supplemented saline testing conditions (lack of sheath and the differences in Hz) are important methodological limitations to this study, and the results should be evaluated accordingly. Load-soak controls were used for each testing condition, which were immersed in serum and saline for the same amount of time as the sample measured from 0–10 million cycles.

Gravimetric Assessment

The methods of assessing gravimetric wear were performed in accordance with ASTM F1714 Standard Guide for gravimetric wear assessment of prosthetic hip-designs in simulator devices and ISO 14242 wear of total hip-joint prostheses. The implants were cleaned and weighed using the following steps.

1. Components were disassembled from mechanical testing machine grips using clean powder-free nitrile gloves.

2. Components were cleaned using a high-pressure water pick.

3. Components were rinsed with deionized, distilled water (DH_2O) and then ultrasonically cleaned for 5 minutes in 2% SDS solution. This was repeated 3 times.

4. Components were rinsed with deionized, distilled water (DH_2O) and then ultrasonically cleaned for 5 minutes in 10 mL detergent + 500 mL H_2O.

5. Components were rinsed with deionized, distilled water (DH_2O) and ultrasonically cleaned in deionized, distilled water (DH_2O) for 10 minutes.

6. Components were rinsed with deionized, distilled water (DH_2O) and ultrasonically cleaned in deionized, distilled water (DH_2O) for 3 minutes. They were then given a final rinse in deionized, distilled water (DH_2O).

7. Components were dried using compressed nitrogen gas and placed in a vacuum desiccator at 27 in Hg for 5 hours.

8. Temperature and relative humidity were recorded.

9. Components were weighed 3 times in rotation using a 01mg resolution balance (Mettler-Toledo, Switzerland).

Particle Analysis

To process the serum-supplemented simulator samples for analysis of polymeric and metallic particles using both low-angle laser light scattering (LALLS) and scanning electron microscopy (SEM) analyses, an enzyme and mild acid digestion procedure was used to provide an environment that was free of bacteria, fungi, protein precipitate, and biofilm particles, to the highest degree.

Enzyme

An initial enzyme digestion with trypsin (2x final concentration: 24 hours at 37°C) was followed by a second enzyme processing stage, wherein 100 µL of 0.5% SDS, 30 mg proteinase K, and 100 µL of Triton X were added to the enzyme digestion sample for 24 hours.

Mild Acid Treatment

Upon completion of the enzyme digestion, the particle-containing semi-processed solutions were centrifuged to 5 mL (2000 rpm for 30 min), and mixed with HCl (initial 37%) at 1 part acid to 1 part sediment and incubated at 37°C for 4 hours, followed by ultra-sonication for > 5 min. The particle-containing acid solutions were mixed with methanol at a 1 to 10 dilution and then washed in ethanol (1000 rpm, 15 min). Following dilution and washing, the particles were resuspended in ethanol or DH_2O (10 mL total volume).

LALLS

Low-angle laser light scattering (LALLS) was used for quantitative number- and volume-based particle analysis. Laser light scattering information obtained from LALLS is only valid within its particle size detection range (Microtac X-100: 0.1 to 2000 microns)(Cabot Corp., Boston, Massachusetts), where the amount of particle debris available for analysis must be greater than approximately 0.02 mm^3 (0.003 mg of Co-alloy debris) to provide enough particles for adequate detection. LALLS works on the principle that diffraction angle is inversely proportional to particle size when passing in front of fixed wavelength He-Ne gas laser ($\lambda = 0.63\mu m$), and thus directly measures millions of particles passing in front of the laser beam to calculate volume and number distribution data. However, it lacks the capability to yield morphologic data, eg, aspect ratios.

Scanning Electron Microscopy (SEM)

SEM using microprobe energy-dispersive X-ray analysis (EDAX) was used to characterize particle shape and chemical composition following the guidelines established in ASTM F1877-05, Standard Practice for Characterization of Particles. The sample containing particles was vacuum-filtered through a polycarbonate filter with 0.1-mm pores (Thermo Fisher Scientific, Waltham, Massachusetts), dried in a filtered desiccator at room temperature for 24 hours, and analyzed in a scanning electron microscope (Hitachi 3000-SN SEM operating at 10-20 kV).

Chemical Analysis

Chemical analysis of the isolated debris was performed using an INCA Energy 200 software program (Oxford Instruments Inc., Oak Ridge, Tennessee) of the energy dispersive X-ray analysis system coupled to the SEM (Hitachi 3000-SN, Hitachi, Tokyo, Japan). X-ray analysis with EDAX is used by the operator to characterize any visibly predominant type of particles (> 10 random particles in 5 fields are analyzed with > 2 EDAX analyses recorded/sample). This facilitates a qualitative assessment of predominant particle type. Polymeric materials produced in protein-containing wear simulators are not generally discernable fro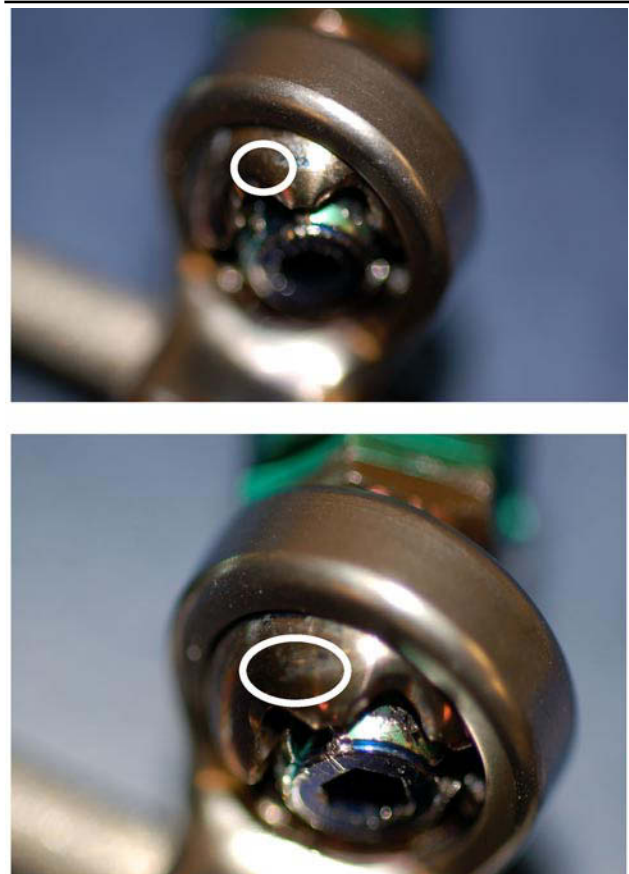m one another under this EDAX analysis assessment due to the variability in protein adsorption and desorption, polymer oxidation in situ, and metal sputter coating required for SEM visualization.

RESULTS

Post-testing Implant Appearance

The appearance of the implants tested in saline and serum demonstrated observable differences (Figures 2 and 3). While the CoCrMo on CoCrMo-articulating surface tested in 100% saline demonstrated significant wear marks (scratches and/or abrasions), the same surfaces of implants tested in serum-supplemented saline maintained a highly polished mirror-finish where the only observable wear marks were circumferential scratches associated with implant placement and removal from the polyethylene pucks at 1 million cycle intervals over 10 million cycles (for cleaning and gravimetric assessment purposes), as shown in Figure 3. Visual inspection of the other contact surfaces in the device in 100% saline (eg, the PTFE sheath connection mechanism, the PTFE sheath itself, and the
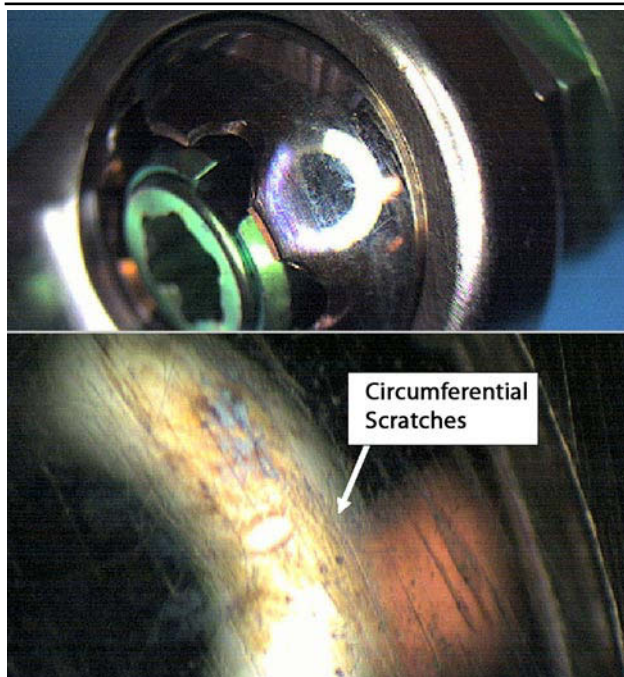
Figure 2.

Saline: Post-simulator testing images of the implant are shown, indicating wear of the CoCrMo/CoCrMo articulating surface(s) after being tested in saline at 37°C, after 10 million cycles. Significant wear marks were evident in the form of scratches and/or abrasions. Visual inspection of the other contact surfaces in the device, namely the PTFE sheath connection mechanism, the PTFE sheath itself, and the cable ferrule sliding contact, did not show any evidence of wear.

cable ferrule sliding contact) did not exhibit observable evidence of wear. The springs were covered, and hence, visual inspection could not be performed. Visual inspection of the surface for implants tested in 20%-serum saline presented very little appearance of wear when compared to implants tested in just saline. SEM analysis (1000x magnification) revealed isolated patches of thin, discontinuous surface deposits and random scratches. The other contact surfaces appeared similar to those observed in specimens tested in 100% saline. These protein deposits were more pronounced within the sheath during initial pilot testing (Figure 4); thus, the sheath was removed for implant testing and gravimetric assessment in the serum-supplemented saline.

Figure 3.

Serum: Post-simulator testing images of the implant are shown, indicating wear of the CoCrMo/CoCrMo articulating surface(s) after being tested in 20% serum at 37°C, after 10 million cycles. Visual inspection of the surface showed very little wear, when compared to implants tested in saline. A highly polished mirror-finish was observed. At high magnification, the most significant wear marks were circumferential scratches associated with implant placement and removal from the polyethylene fixture pucks 10 times over 10 million cycles (at 1 million cycles intervals), for cleaning/weighing purposes. SEM analysis (1000x magnification) revealed isolated patches of thin, discontinuous surface deposits and random scratches. The other contact surfaces appeared similar to those observed in specimens tested in saline.

Gravimetric Analysis

There was an average of > 20-fold increase ($P < .05$) in the weight loss of the dynamic stabilization implant when tested in all saline (undetectable) when compared to 20%-serum-supplemented conditions (Figure 5). After 10 million cycles of testing, the gravimetric weight loss was approximately 200 mg for the implants tested in saline with approximately 20 mg of wear every 1 million cycles.

Figure 4.

Protein deposits were demonstrated within the protective sheath around the spring assembly during initial pilot testing and necessitated the removal of sheath during subsequent testing in serum.

The implant samples tested in 20% serum were below the method detection limits (< 10 mg over 10 million cycles). There was a linear rate of weight loss for implants tested in saline over the 10 million cycles, with approximately 20 mg/million cycles of wear which corresponds to a volumetric loss of approximately 2.5 mm^3/million cycles. This is in stark contrast to the seeming "weight gain" of the same implants tested in 20% serum (Figure 5). Despite the thorough cleaning of the implants when tested in both saline and 20% serum, there was measurable weight gain associated with serum testing, over the course of 10 million cycles, at a linear rate of 1 mg/million cycles. The source of this weight gain was not identified. The rate of 1 mg/million cycles and 10 mg/10 million cycles of weight loss was below the level of standard deviation for all implants tested and was not significantly elevated above load-soak controls (and thus below method detection limits).
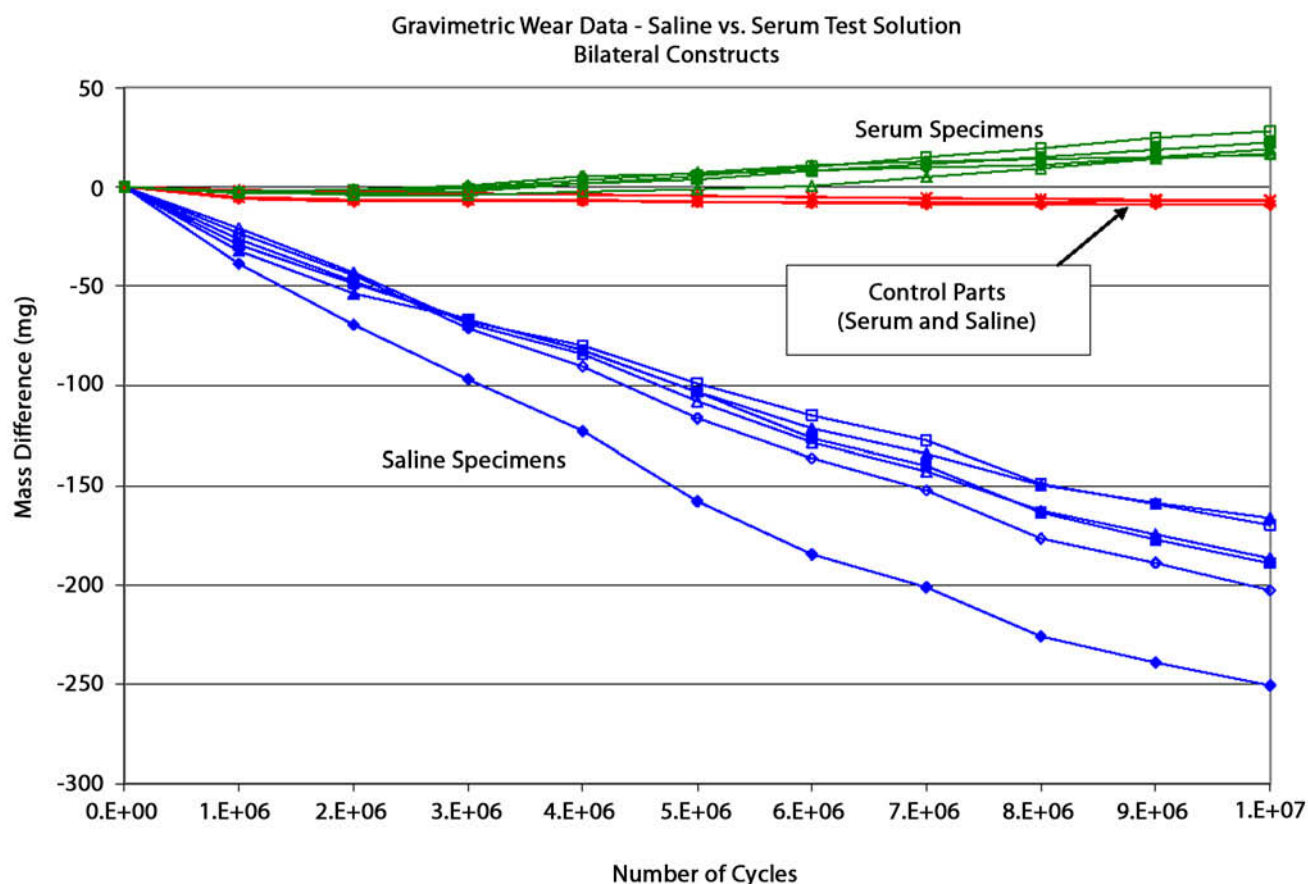
Load-soak controls used to test for any weight gain or loss due to corrosion or protein adsorption did not demonstrate any measurable weight gain or loss after equal immersion time of 10 million cycles. The controls were not statistically different in weight than implants tested in 20% serum up to 10 million cycles, indicating that protein build-up was likely not the result of passive adsorption onto the exposed external surfaces on the implants.

Particle Analysis
LALLS
The simulator fluids of each sample were analyzed at 1 million cycle intervals over the course of 10 million cycles for each of the 12 implants tested in 6 stations of saline and 6 in 20%-serum saline. Particle size was measured using laser diffraction analysis (LALLS), which generated number- and volume-based distributions (see Figure 6). Average size data provided by LALLS analysis was based on an equivalent spherical diameter of millions of sampled particles passing in front of a laser and detector. The number distributions represent the size distribution as a percentage of the total number of particles, and the volume-based analysis/distributions represent the size distribution as a percentage of the cumulative (total) volume of particles.

Figure 5.

Gravimetric analysis of implant weight shows the weight loss (and gain) of the implants when tested in 100% saline and 20%-serum saline, with an average of > 20-fold increase (*P* < .05) in the weight loss of the dynamic stabilization implant when tested in saline only when compared to 20% serum-supplemented conditions (after 10 million cycles).

The particle distributions of the saline and 20% serum fluids demonstrated markedly different patterns (Figure 7). The particles produced in saline were relatively consistent in size and distribution, and extended from 0.1 to 100 μm in size (Figure 7). The particle distributions produced in 20% serum demonstrated a change in the distribution pattern of debris after 3 million cycles, where a wide "bell-shaped" distribution was followed by a more discontinuous distribution indicative of less debris after a "wear-in" period.

Compiling the results for all 6 specimens in saline and all time points, the mean particle diameter was 16 microns (range 0.5–85 microns on a volume basis). When re-analyzed in the same manner (LALLS) but based on particle percentage of total number of particles rather than volume, the mean particle diameter was 0.22 microns (range 0.14–0.55, on a number basis), see Table 2. Average volume and number particle size distributions are shown in Figure 8, where the difference in the average size of the particles, 0.2 μm and 4 μm (mn, number-based analysis) in 100% saline and 20% serum, respectively, are illustrated.
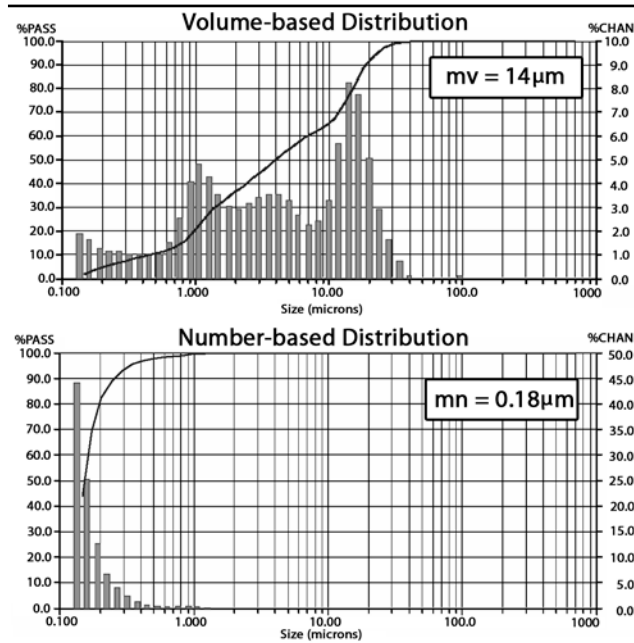
The volume analyses (Figure 8) show a similar difference between the saline and the 20% serum, ie, 16 μm vs. 39 μm, respectively (mv, volume-based analysis).

For both 100% saline and 20%-serum simulator fluids, the average particle size over the course of 0 to 10 million cycles remained relatively constant (Figure 9). The size of the generated particles in saline remained relatively constant at 20 microns mv and 0.1-0.2 microns mn (saline), and 48 microns mv and 3 microns mn (20% serum). There was a slight, non-significant increase in the size of the particles over the course of testing. This would tend to indicate that the mechanism of wear debris generation remains relatively constant over the course of 10-million cyclic load.

SEM
There were gross differences between debris particles produced in 20% serum and 100% saline (Figures 10, 11 and 12). Particles produced in saline and analyzed by SEM and chemical analysis were flake-like and granular in shape and primarily chromium oxide. Particles greater than 5 μm

Figure 6.

The particle size distributions of a single sample are shown. Particle size analysis using laser diffraction analysis (LALLS) can generate a number-based and a volume-based distribution, because of the millions of particles analyzed. Average size data provided by LALLS analysis was based on an equivalent spherical diameter of millions of sampled particles passing in front of a laser and detector. The number distribution represents the size distribution as a percentage of the total number of particles and the volume-based analysis/distribution represent the size distribution and a percentage of the cumulative (total) volume of particles. The percentage of particles within each size range is represented by the bars (right axis) and the cumulative percentage of total particles below any size is indicated by the line (left axis).

were generally flake-like in morphology while the smaller particles < 5 µm were generally granular, where both had 2D average aspect ratios of between 1 and 2 (aspect ratio = length/width) (Figure 10). Less than 1% of the particles in saline were polymeric (from the polymeric sheath or fixation blocks). Due to the lack of particles in the 20%-serum solutions, polymeric contamination from fixation grips was estimated to be increased to < 60% in 20% serum. Using an SEM analysis of the number of particles within 5 similar fields, there was approximately > 1000x more particles generated in saline than in 20% serum. EDAX analyses of particles demonstrated that the saline debris was Cr, Co, P, and O in composition (orthophosphate-like oxides), and for 20% serum metal Co and Cr was detected (Figure 11). The Cr-O composition of the debris was characteristic of fretting corrosion. The smaller particles (< 5 microns) tended to contain more Cr than larger particles, which contained more surface Co. The metallic particles identified in the 20% serum were cobalt and chromium in composition, indicative of abrasive wear of the implant surface and bulk alloy (Figure 12).

DISCUSSION

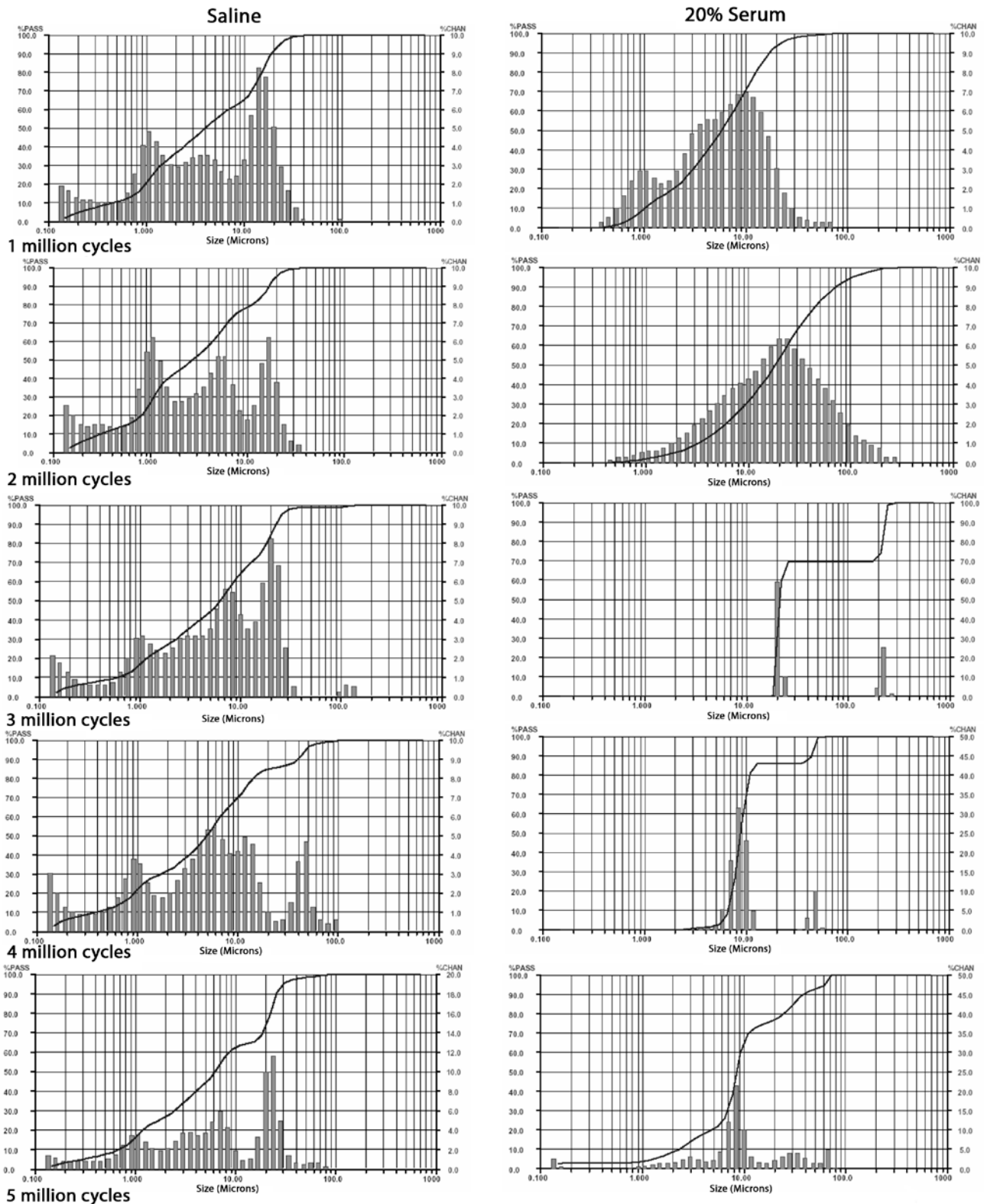Our initial hypothesis, that 100% saline versus 20% serum simulator lubricants would not cause a significant difference in the amount of gravimetric weight loss, was not supported by the data when tested using a spinal dynamic stabilization implant with metal-on-metal bearings. On the contrary there was greater than 100-fold increase in the amount of wear generated in the saline-only when compared to the 20% serum, as determined by weight loss, ie, gravimetrically. In addition, there were marked differences in the average size and the type of wear debris that were directly and mechanistically attributable to the type of simulator fluid used, where smaller particles of chromium oxide were evident in the saline simulator fluids when compared to serum (Average sizes: Serum = 3.2 microns diameter, Saline = 0.2 microns diameter), indicative of fretting corrosion. Saline resulted in high amounts of released Co-Cr oxide debris indicative of a fretting-like corrosion phenomenon.

Fretting corrosion generally occurs at modular junctions (connections) of any loaded orthopedic implant to some degree and is typically produced by relatively small-scale (1 – 100 µm) motion between implant components induced by cyclic loading. However, all that is required to produce fretting type corrosion is the continual fracture and reformation of oxide layers (repassivation), which form over metal surfaces of implants (or any metal in a corrosive environment) causing a O_2-depleted micro-environment, thereby accelerating corrosion reactions within the crevices or any location where oxygen reduction can be maintained. This process is driven by the reduction in free energy associated with ceramic layer forming on the metal surface (metal-oxide formation) which results in the sequestration of O2 from any available source (primarily H_2O).[9,10]

Since implant fretting corrosion was first identified in the late 1950s,[11,12] many investigations have characterized fretting of implant materials.[9,13-22] Past studies have shown markedly different rates of fretting associated with saline and proteinaceous solutions.[23,24] Generally, decreased rates of fretting corrosion have been observed in proteinaceous solutions when compared to levels of fretting weight loss in physiologic saline solutions, similar to the results found in the current investigation.

Thus testing in pure saline likely produced an artificially high amount of corrosion/wear debris (weight loss), compared to what would likely occur in vivo where generally 100% serum conditions exist. To date there are no clinical reports of wear rates in dynamic stabilization implants. It remains unknown how the "protective" effect of serum proteins at the ASTM standard of 20% of the simulator lubricant will affect the wear of this metal-on-metal articulating spinal implant and how it would vary at increasing concentrations up to 100%. However, the weight gain witnessed in this study of a highly modular component is likely the consequence of protein and/or liquid penetrating tight junctions of the component that generally

Figure 7.

The volume-based distributions of LALLS particle analysis, shown here, demonstrate the different distribution patterns of saline and 20% serum fluids. The particles produced in saline were relatively consistent in size and distribution, and extended from 0.1 to 100 μm in size. The particle distributions the debris produced in 20% serum demonstrated a change in the distribution pattern of debris after 3 million cycles, where a wide bell-shaped distribution was followed by a more discontinuous distribution indicative of less debris after a wear-in period. These graphs are meant to highlight the general shape changes in the distributions over the course of testing from 1 to 5 million cycles.

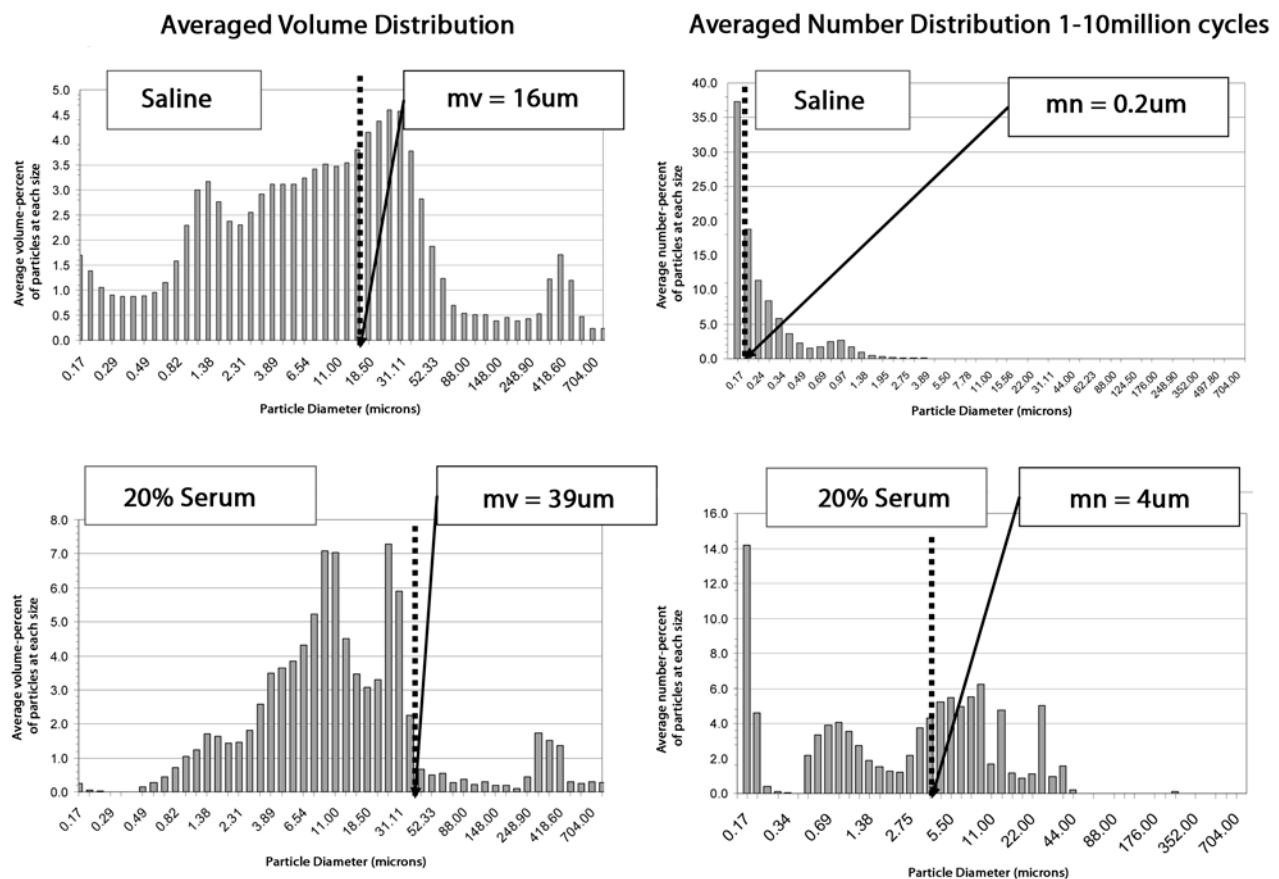**Table 2.** Gravimetric Weight Loss and Wear Debris Particle Sizes (Over 10 Million Cycles of Simulated Loading)

Fluid	Weight Loss (mg)	Type of Debris (EDAX)	Average Diameter (Volume)	Average Diameter (Number)	Average Volume Percent of Small (Phagocytosable) Particles		
					<1µm	<2µm	<5µm
Saline/ Serum	Not detectable	Co-Cr alloy	48.4µm	3.2µm	2.6%	6.2%	17.6%
Saline	200	Cr-Co-Oxide	16.2µm	0.2µm	17.8%	28.7%	44.0%

accumulate over the course of testing. This was indicated by the lack of weight gain in the load-soak controls where after immersion time equivalent to 10 million cycles there was no indication that protein build-up was the result of passive adsorption onto the exposed external surfaces on the implants, but instead was likely due to protein build-up within crevices/modular connections available for protein deposition only during cyclic loading of the implants. These phenomena of weight gain, protein

adsorption, and crevice accessibility during simulator testing will likely become more common with increasing numbers of multicomponent implant designs. Ultimately the end result of this protein/fluid accumulation in modular junctions may mask wear/corrosion degradation that takes place during the gravimetric weighing of the implants over the course of testing.
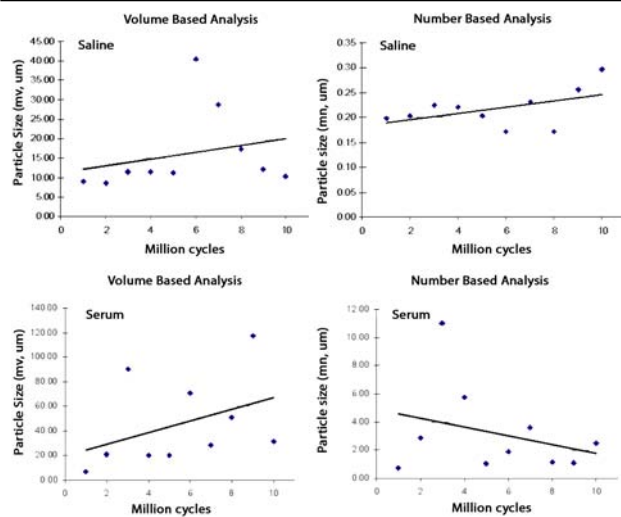
The results of this study illustrate the modern testing predicament in the assessment of wear/corrosion of a multicomponent spinal implant using in vitro mechanical simulation. While pure saline produced non-physiologically high amounts of wear/corrosion debris, the addition of serum (20%) resulted in masking any wear/corrosion that took place. It is likely that testing should be conducted at a non-ASTM and -ISO standard level of serum protein less than 20%. The exact composition would likely vary from implant to implant, where geometrical considerations may be more or less dominant. However, when assessing the physiologic importance of wear/corrosion testing, pure
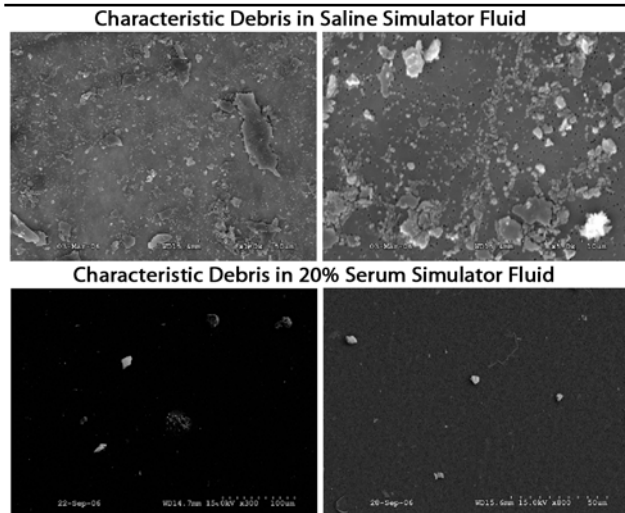
Figure 8.

The average volume and number particle size distributions are shown, where the difference in the average size of the particles were 0.2 µm and 4 µm (mn) in in 100% saline and 20%-serum saline, respectively, are illustrated. The volume-based distributions (analyses) show a similar difference between the saline and the 20% serum, ie, 16µm vs. 39µm, respectively.

Figure 9.

The graphical analysis of volume and number based representations of particle size showed relatively constant average particle size for both saline and 20% serum simulator fluids, over the course of 0 to 10 million cycles. The size of the generated particles in saline remained relatively constant at 20 microns mv and 0.1-0.2 microns mn (saline) and 48 microns mv and 3 microns mn (20% serum). The non-significant increase in the size of the particles over the course of testing indicates that the mechanism of wear debris generation remains relatively constant over the course of 10 million cycles of load.
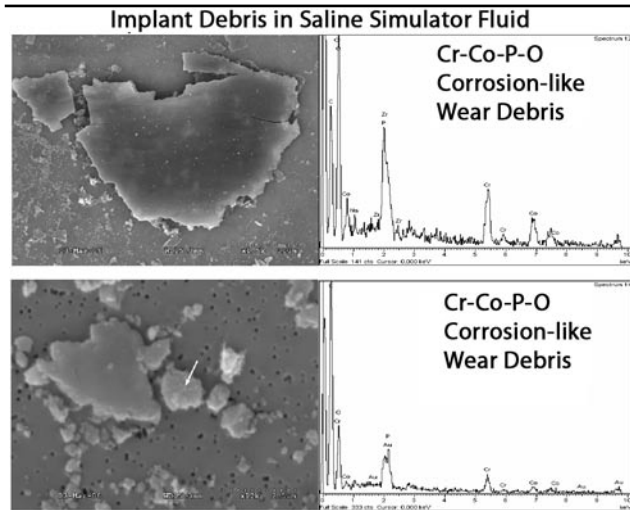
Figure 10.

Particles produced in saline and analyzed by SEM and chemical analysis were flake-like and granular in shape and primarily chromium oxide. Particles greater than 5μm were generally flake-like in morphology while the smaller particles < 5 μm were generally granular. Less than < 1% of the particles in saline were polymeric (from the polymeric sheath or fixation blocks). Due to the lack of particles in the 20% serum solutions, polymeric contamination from fixation grips was estimated to be increased to < 60% in 20% serum. Using a SEM analysis of the number of particles within 5 similar fields, there was approximately > 1000x more particles generated in saline than in 20% serum.
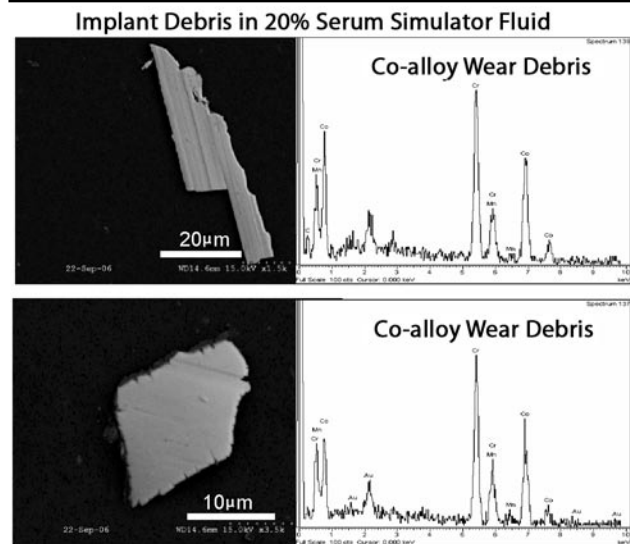
saline clearly represents a non-physiologic "worst case" scenario, when compared to the in vivo data from metal-on-metal total hip and disc arthroplasties since clinical data for this implant is not currently available. However

Figure 11.

Scanning electron microscope (SEM) analysis using X-ray (EDAX) microprobe analysis of implant debris particles produced in saline simulator fluids demonstrated the prevalence of particles consisting of Cr, Co, P, and O in composition (orthophosphate-like oxides). The Cr-O composition of the debris was characteristic of fretting corrosion-like products, where smaller particles (< 5 microns) tended to contain more Cr than larger particles which contained more surface Co.

Figure 12.

Scanning electron microscope (SEM) analysis using X-ray (EDAX) microprobe analysis of implant debris particles produced in 20% serum simulator fluid demonstrated the prevalence of particles consisting of Co-Cr in similar proportion to the bulk alloy. The metallic particles identified in the 20% serum were cobalt and chromium in composition indicative of abrasive wear of the implant surface and bulk alloy.

the degree to which results of testing in pure saline can be appropriately scaled down to approximate in vivo conditions is not known and leaves open the question of "to what degree do the results of testing in pure saline (the only conditions to produce significant degradation) relate to in vivo degradation and implant performance?" The only definitive answer to this question lies in the evaluation of available retrieved implants after years of clinical use.

However, one way to assess the performance of modular spinal implants with metal-on-metal articulation in saline (worst case) is to compare them to the results of other metal-on-metal implants.

Wear is generally measured gravimetrically, using the weight loss of an implant over the course of testing. However, because of density variations between materials, the amount of wear between different implants is compared using a volumetric wear basis (adjusting the mass loss to volume using the average density of the implant materials). Because of the different implant geometries and surface areas, linear penetration, as is used in some studies, is an inappropriate measure of wear and when comparing different types of implants. Also important to wear characteristics of a particular implant is the average size of the debris particles, as the debris size will determine the numbers of particles that are generated from a given amount of wear volume. Generally the greater the numbers of particles the greater the untoward biologic response will be.

There are relatively few published reports on the in vivo wear rates of articulating spine implants in general, and even fewer report of those with metal-on-metal articulating surfaces to compare the results of the current investigation to.[4,5,25] However, a recent in vitro study of the metal-on-metal total disc arthroplasty implant by Pare et al using a spine simulator (flexion-extension ±10° at a constant load of 1200 N in 11.5 g/L protein up to 10 million cycles) found a volumetric wear rate of 1.26 mm^3 per million cycles (Figure 13).[25] Other similar studies of estimated total wear volume of an intervertebral disc prosthesis with an all titanium-6%Al-4%V alloy was reported to be 2.9 mm^3/million cycles.[4] The volumetric rate of "worst case" wear/corrosion using saline in this study was 2.5 mm^3/million cycles, which was less than that reported for Ti-alloy spinal components but more than that reported for a cobalt alloy metal-on-metal disc arthroplasty implant tested in serum-supplemented saline.[25] The lack of measurable wear in 20% serum of the dynamic stabilization device in this study implies that factors such as less direct loading and decreased articulation (sliding) when compared to total disc arthroplasty implants may result in less wear, if other factors like fretting corrosion do not become dominant in vivo.
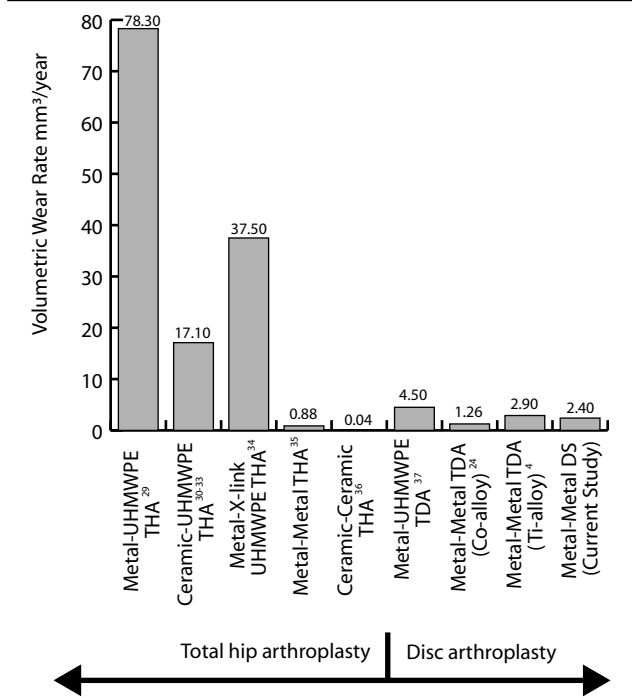
Inflammatory response to particles is generally determined by the particle load (ie, number of particles). This load or number of phagocytosable particles released is determined by both the average size of the particles (smaller particle size = more particles for a given volume loss) and total volume of wear debris (the more volume lost = more particles of a given size).[26,27] If a given amount of debris is comprised of smaller diameter particles (within the 0.1-10 µm range) it is by virtue of their greater numbers

per volume that they induce more of a proinflammatory response than the larger particulates.[27] However on a particle-to-particle basis (ie, equal numbers of the large and smaller size particles), the degree to which smaller or larger particles will induce a response has not been thoroughly investigated and remains unknown. What is clear is that the numbers of particles produced by the current implant in saline would have a far greater biologic response than the particles produced in serum, by virtue of their vastly greater numbers at the same cycle counts (roughly at least a > 10^6-fold increase in numbers of particles).

Another important component of particle characterization is the degree to which large particles comprise the total volume of the debris. Large (> 10 µm) particles were evident in both fluid environments demonstrating the importance of using a particle characterization method such as LALLS which samples millions/billions of particles to statistically "catch" the larger size particles that contribute significantly to the overall volume or weight loss but are not observed on a number distribution because the make-up is a negligible percent of the total number of particles. Identifying the presence of large particles within a given amount of debris effectively reduces the overall particle burden (number) because larger particles make up a disproportionately high percentage of the total volume. A single 100 micron diameter particle equals the same volume as 1 million 1 micron diameters particles. So identifying just how much of the total volume of debris is comprised of larger particles is important to calculating how many particles were generated from a given implant for a given amount of volume loss.

It is generally established that the related phenomena of aseptic loosening and periprosthetic osteolysis (the most common reasons for revision surgery) are a consequence of the host response to the amount of debris originating from prosthetic devices (numbers of particles).[28] The majority of this debris originates from articulating surfaces. For the current implant, the results of the average distributions of particle sizes over 10 million cycles (volume-based distributions to factor in larger particles) were used to calculate the total number of particles generated which was approximately 2.5x10^8 particles/mg (2 x 10^9 particles/mm^3) of Co-alloy debris and equates to approximately 485 x 10^8 particles per 10 million cycles (average weight loss was 194 mg). This amount of wear debris (194 mg or approximately 24 mm^3 of lost volume) corresponds to approximately 2.4 mm^3 of lost volume per 1 million cycles (or per year based on a an assumed cycle load of 1 million bends per year). This is within the range of other spine arthroplasty implants (Figure 13)[4,29-36] and well below the wear of other metal-on-metal or metal-on-polymer total joint arthroplasty implants (Figure 13).[29-36]

Figure 13.

A graphical comparison of data showing the amount of wear debris generated in the current study (Metal-Metal DS) of a dynamic stabilization implant tested in saline (without the corrosion/wear reduction of serum protein) to wear rates of past investigations of other spine and total joint arthroplasties. This comparison demonstrates the similar range of wear to other TDA implants and the 10-fold decrease in debris compared to metal-on-polymer THA articulation.

CONCLUSIONS

There were significant differences in gravimetric wear, average size and type of wear debris that were mechanistically attributable to the type of simulator fluid used (ie, corrosion versus abrasive wear). Large (> 10 μm) particles were evident in both fluid environments demonstrating the importance of non-number based analysis methodologies. Pure saline resulted in non-physiologically high Cr-Co fretting-like corrosion debris and 20% serum resulted in Co-Cr debris that was below gravimetric method detection limits (10 mg over 10 million cycles). This highlights the practical limitations in simulator testing and the importance of interpreting wear and corrosion data in the appropriate context, ie, testing conditions. The overprotective effect of serum proteins appears to underscore the importance of using both saline and serum when establishing upper and lower bounds of predictive implant debris generation modeling, where saline represents a worst case scenario and as little as 20% serum masks all weight loss completely. The fact that serum protein adsorption can mask wear suggests that wear testing highly modular, articulating spine implants can be problematic, especially under conditions of low but significant wear. Simulator testing conducted in serum alone may not adequately indicate actual weight loss or debris generation, which may translate to more debris

released clinically than predicted. Thus, care should be taken in the interpretation of results of modular articulating implants tested in serum-supplemented simulator fluids.

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