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In Vitro Evidence for Cell-Accelerated Corrosion within Modular Junctions of Total Hip Replacements

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Abstract

Corrosion at modular junctions of total hip replacement (THR) remains a major concern today. Multiple types of damage modes have been identified at modular junctions, correlated with different corrosion characteristics that may eventually lead to implant failure. Recently, within the head-taper region of the CoCrMo retrieval implants, cell-like features and trails of etching patterns were observed that could potentially be linked to the involvement of cells of the periprosthetic region. However, there is no experimental evidence to corroborate this phenomenon. Therefore, we aimed to study the potential role of periprosthetic cell types on corrosion of CoCrMo alloy under different culture conditions, including the presence of CoCrMo wear debris. Cells were incubated with and without CoCrMo wear debris (obtained from a hip simulator) with an average particle size of 119 ± 138 nm. Electrochemical impedance spectroscopy (EIS) was used to evaluate the corrosion tendency, corrosion rate, and corrosion kinetics using the media after 24h of cell culture as the electrolyte. Results of the study showed that there was lower corrosion resistance ($p < 0.02$) and higher capacitance ($p < 0.05$) within cell media from macrophages challenged with particles when compared to the other media conditions studied. The potentiodynamic results were also in agreement with the EIS values, showing significantly higher corrosion tendency (low E_{corr}) ($p < 0.0001$) and high I_{corr} ($p < 0.05$) in media from challenged macrophages compared to media with H_2O_2 solution. Overall, the study provides *in-vitro* experimental evidence for the possible role of macrophages in altering the chemical environment within the crevice and thereby accelerating corrosion of CoCrMo alloy.

Graphical Abstract

This study has successfully established an *in-vitro* test protocol to characterize the cell accelerated corrosion behavior of CoCrMo alloy in conditioned cell culture media of different periprosthetic

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Author contributions

A, designed, directed, performed experiments, analyze and interpret the data, and write the first draft of manuscript. B, performed experiments, analyze the data and help to write first draft of the manuscript. C, performed electrochemical experiments. D, Performed material characterization and flow analysis, E, aided in interpreting the results and worked on the manuscript. F&G, conceived the original idea, were in-charge of overall direction and planning, and contributed in final version of manuscript.

Conflict of interests

The authors confirm that there is no conflict of interest.

cell types. Macrophages activated with wear particles had a significant effect on the increased corrosion rate and the overall corrosion kinetics. Thus, macrophages can alter the electrochemical environment that drives the passivation and preferential dissolution processes at the microstructure level of the alloy, thus accelerating damage caused by corrosion.

Keywords

macrophages; periprosthetic cells; corrosion; CoCrMo; electrochemical impedance spectroscopy (EIS); modular junction; THR

1.0. Introduction

Fretting and corrosion within modular taper junctions of total hip replacements (THR) is a persisting problem in orthopedics. Adverse local tissue reactions (ALTR) due to tribocorrosion debris is recognized as a frequent cause of premature implant failure¹⁻³. Reports of the prevalence of ALTR related failures vary between 2 and 4%^{4,5}. Considering the increasing number of THRs performed annually, it is important to reduce this number by preventing the occurrence of fretting and corrosion processes. A unified model of fretting and corrosion within modular junctions has been described as mechanically assisted crevice corrosion (MACC)⁶. The crevice environment is given by the interface between the head and stem taper surfaces. The mechanical aspect is primarily provided by micro-motion that leads to fretting wear and corrosion. Yet, retrieval studies have shown that the resulting damage cannot be explained by one single damage mode⁶⁻⁹. Multiple underlying damage modes occur which can be either mechanically or chemically driven⁹. Many *in vitro* fretting studies have been conducted to simulate *in vivo* fretting processes¹⁰⁻¹³. To our knowledge, no study was able to recreate all damage features that have been observed *in vivo*. Specifically, chemically dominated damage features such as etching, pitting, intergranular corrosion, and phase boundary corrosion have not been reproduced using a fretting apparatus. Therefore, it appears that the chemistry of the environment is a significant factor that needs to be considered for *in vitro* testing. Gilbert et al. presented a model of inflammatory cell-induced corrosion suggesting that reactive oxygen species released by macrophages or other cell types may cause corrosion damage to the implant surface¹⁴. However, so far there is little evidence that macrophages or other cell types of the joint environment could significantly contribute to the corrosive process within THR modular taper junctions.

The hypothesis of this study was that among periprosthetic cell types, macrophages will have the most dominant impact on the generation of a corrosive environment under inflammatory conditions. Hence, the major goal of this study was to generate *in vitro* experimental evidence for cell-accelerated corrosion by creating *in vivo*-like conditions in lab-scale and develop an *in vitro* model for the evaluation of the molecular mechanisms of cell-mediated corrosion.

2.0. Materials and methods

2.1. Overview of sample groups

The investigation of the corrosion behavior of CoCrMo alloy under normal and inflammatory media conditions was carried out by means of electrochemical analysis. Three different cell types (cell lines) from the periprosthetic environment—monocytes, macrophages, and osteoblasts—were used in this study. Normal medium condition (conditioned medium) was the medium collected after 24 h of cell culture (monocytes, macrophages, or osteoblasts). Inflammatory medium conditions were generated by challenging cell cultures with CoCrMo wear debris. The viability of cells under inflammatory conditions was evaluated by cytotoxicity and particle uptake analysis. Cell culture media from both normal and inflammatory conditions were employed as an electrolyte in an electrochemical corrosion chamber. The corrosion behavior of CoCrMo alloy within different electrolytes was determined by open circuit potential (OCP) measurements, cyclic potentiodynamic polarization tests, and electrochemical impedance spectroscopy (EIS) (Figure 1).

2.2. Sample Material and Preparation

Low carbon wrought CoCrMo alloy (Carpenters Ltd, CA, USA) as specified by the ASTM¹⁵ was used to prepare sample discs 12 mm in diameter with a thickness of 7 mm. These discs were used for both the corrosion tests and the generation of wear particles to challenge cells in culture. For the corrosion tests, the sample surface was mechanically ground using 320 grit silicon carbide grinding paper. This rougher surface finish was chosen to resemble head taper surfaces¹⁵ more closely. Prior to testing all samples were ultrasonically cleaned in 75% 2-propanol and distilled water for a duration 15 minutes each. For wear particle generation, the prepared discs were polished to a mirror finish.

2.3. Wear particle generation

In order to generate inflammatory conditions in the cell culture tests CoCrMo particles with a known size range were added to the different types of cell cultures. These particles were generated using a tribocorrosion test rig with a pin-on-ball configuration¹⁶ (Figure 2a). The experimental conditions were previously developed and optimized by Mathew et al^{17,18} (See Supplementary file). In order to determine the wear particle size range and chemical composition, dynamic light scattering (DLS; Zeta sizer, Nano ZS, Melvern instruments GmbH, Germany) and scanning electron microscopy (SEM, Hitachi SU8030 FE-SEM, Tokyo, Japan) with energy dispersive x-ray spectroscopy (EDS) were employed.

2.4. Cell Culture

2.4.1 Cell lines—The THP-1 human monocytes cell line (ATCC, Virginia, USA) was cultured in RPMI 1640 supplemented with L-Glutamine, penicillin & streptomycin (100IU/ml), 25 mM Hepes buffer and 10% fetal bovine serum. Cells were subcultured in 1:3 ratio after 80% confluency. RAW 264.7 (mouse macrophage cell line), and MG63 (human osteosarcoma cell line) were obtained from ATCC. Both RAW 264.7 and MG-63 cell lines were maintained in DMEM supplemented with L-glutamine and (Gibco, USA), 10% FBS

(Sigma Aldrich, USA) and 100IU/ml penicillin–streptomycin under standard conditions. Cells were detached from the culture plate at 80–85% confluency. Once confluent, they were passaged in a 1:3 ratio.

2.4.2 Cytotoxicity—The cells (monocytes, macrophages, and osteoblasts) were seeded onto 96 well plates at a seeding density of 5000 cells/well. Wear particles at concentrations in the range of 10 g/ml to 0.1 g/ml were added to cell cultures for 24 h of incubation. In a previous study by Bijukumar et al., we observed that concentrations above 10 g/ml caused a reduction in cell growth to less than 40% in the case of neuronal cells¹⁹. Hence, in this experiment, we selected concentrations from 10 g/ml to 0.1 µg/ml to evaluate their effect on the cells of interest in this study. The aim of this test was to identify the concentration of wear particles showing lower growth inhibition (IC₅₀) after 24h compared to control in order to challenge the cells. After 24 h, the cells were incubated with 10% alamarBlue (Invitrogen, USA) in complete medium for 4 h. After incubation, the optical density at 570nm with 600nm as reference was recorded using a microplate.

2.4.3 Determination of particle uptake—To study the cellular uptake of particles 50,000 RAW, THP1 and MG63 cells were seeded in 12 well plates. Each type of cells was treated with 1 µg/ml of wear particles. Controls of each type were maintained containing the same number of cells in the plain medium. All experiments were conducted in triplicate. The plates were incubated for 24h, after which the cells were trypsinized, collected, and vortexed to obtain a single-cell suspension. The percentage of the cell population that had taken up the particles were analyzed using a flow cytometer (BD FACS Calibur, USA). In the flow cytometer, a laser light beam of 499nm illuminated the cells in the sample stream. The forward and side scatter light intensities were considered to be proportional to the size of the cells and the granularity of the cells, respectively.

2.5. pH measurement

The pH of the media collected for the experiments were measured just before the electrochemical experiments. The control medium, medium with wear particles (freshly prepared) and the medium with H₂O₂ (freshly prepared) were taken from the refrigerator and warmed to 37° C in a water bath using a closed container. Conditioned medium and medium from the cells challenged with wear particles after 24h (already in 37 °C incubator) were collected in a closed container, and the pH was also measured just before the electrochemical testing. The time duration between the collection of samples and pH measurements was 15 to 20 minutes.

2.6. Electrochemical tests

CoCrMo samples were mounted onto a custom-made poly sulfonate multi-well corrosion chamber which exposed an area 0.95 cm² of the sample surface²⁰ (Figure 2b). Alloy samples were connected to a single channel of an electrochemical multiplexer, which was controlled by a Gamry interface 1000E potentiostat in a 3-electrode configuration. The setup included a reference electrode (SCE), a counter electrode (graphite rod), and the CoCrMo alloy sample served as the working electrode. The sequence of analysis included the monitoring of the open circuit potential (OCP), a potentiostatic scan (PS), repeated OCP

monitoring, electrochemical impedance spectroscopy (EIS), cyclic potentiodynamic polarization (CP) and a final monitoring of the OCP. Measurements were conducted at 37 °C using different electrolytes. Electrolytes included cell culture media used for the three different types of cell cultures conducted under normal and inflammatory conditions. Additionally, several controls were used: 1) pure cell culture medium, 2) cell culture medium that was incubated with cells for 24h (conditioned medium), 3) cell culture medium that was incubated for 24h with the addition of 1µg/ml of wear particles, 4) cell culture medium with a 0.03M concentration of H₂O₂ (Sigma Aldrich, USA). The latter was tested as it was suggested to be one of the active agents released by macrophages under inflammatory conditions^{14,21}.

The potentiodynamic test was performed from -0.8 V to 1.8 V vs. SCE with a scan rate of 1mV/s. After the experiment, OCP, EIS, and potentiodynamic polarization data were used to analyze the corrosion tendency, corrosion rate, and corrosion kinetics. The corrosion tendency (I_{corr}) and corrosion potential (E_{corr}) were obtained from the potentiodynamic data. EIS tests were performed at OCP at the frequency from 100 kHz to 0.01 Hz with an amplitude of $\pm 10\text{mV}$. A modified Randle's circuit was used as an equivalent electrical circuit to model the EIS data from which resistance to polarization (R_p) and capacitance (C_{dl}) were obtained. All experiments were repeated three times ($N=3$), and all media used in the three experiments were generated in three independent cell culture experiments. Normality of the data from electrochemical tests was confirmed with QQ-plots. If data was normal, a one-way ANOVA was used with Bonferroni correction (significance level $p=0.05$). If data was not normal, a Kruskal-Wallis test was conducted, followed by a Mann-Whitney U test for pairwise comparison against control.

3.0. Results

3.1. Wear particle characterization

The DLS spectrum showed that the CoCrMo wear particles ranged from the nanoscale to the microscale with an average particle size of $119 \pm 138.4 \text{ nm}$ (Figure 3a) with a median value of a 164.2nm and interquartile range (Q3-Q1) of 399.93nm (1st Quartile - 58.77 and 3rd Quartile - 458.7). Sixty-five percent of particles were under 150nm in diameter. SEM and EDS analysis of larger particles and/or particle agglomerates revealed the presence of both Co and Cr species within the particles (Figure 3b, c).

3.2. Cytotoxicity and wear particle uptake analysis

Cell cultures were incubated with different wear particle concentrations (10 µg/ml, 5 µg/ml, 1 µg/ml, 0.1 µg/ml) for a period of 24 h in order to optimize the dose required to challenge cell cultures without causing necrosis due to toxicity. The results showed that higher concentrations of wear debris (5 and 10 µg/ml) were toxic to all tested cell types after 24 h. In the case of 10 g/ml concentration of wear particles, cell viability was reduced by $48.9 \pm 2.6\%$ ($p=0.0008$), $13.9 \pm 2.1\%$ ($p=0.025$), and $39.2 \pm 1.41\%$ ($p=0.005$) in osteoblasts, macrophages, and monocytes respectively. Similarly at 5 g/ml the reduction in cell viability was observed as $31.4 \pm 2.3\%$ ($p=0.001$), $14.2 \pm 4.1\%$ ($p=0.112$), and $30.5 \pm 2.5\%$ ($p=0.02$) in osteoblasts, macrophages, and monocytes respectively. There was no significant toxicity

observed at 0.1 $\mu\text{g/ml}$ [osteoblasts $95.1 \pm 3.2\%$ ($p=0.125$), macrophages $100.9 \pm 2.2\%$ ($p=0.55$) and monocytes $97.33 \pm 6.1\%$ ($p=0.66$)] and 1 $\mu\text{g/ml}$ [osteoblasts $88.5 \pm 5.7\%$ ($p=0.125$), macrophages $104.9 \pm 5.5\%$ ($p=0.55$) and monocytes $97.1 \pm 2.4\%$ ($p=0.27$)] (Figure 4a). Furthermore, flow cytometry confirmed the cell uptake of wear particles at 1 $\mu\text{g/ml}$ concentration (Figure 4b). The side scatter (Q_4) vs. forward scatter (Q_2+Q_3) plots illustrated that cellular granularity increased over time, which suggest that the cells had readily taken up the wear particles. However, from the cytogram and the corresponding bar graph, it was clear that particle uptake was higher in macrophages compared to monocytes and osteoblasts. In addition, the forward scatter of macrophages was significantly ($p < 0.001$ for challenged monocytes vs. challenged macrophages and $p < 0.0004$ for challenged osteoblasts vs. challenged macrophages) higher suggesting that the increase in cell size occurred due to the presence of wear debris.

3.3. Electrochemical analysis

3.3.1 pH variation—The pH of the different media such as control medium, DMEM medium from 24h cell culture of macrophages, and DMEM medium with H_2O_2 was analyzed just before the experiment using a pH meter (Table 1). All media conditions except medium collected after 24 h of incubation of macrophages with wear particles had an average pH in the range of 7.30 ± 0.07 (conditioned medium) to 7.62 ± 0.01 (DMEM with $0.03\text{M H}_2\text{O}_2$). However, in the case of challenged macrophages, the pH value was slightly acidic at 6.74 ± 0.14 (Table 1).

3.3.2. Open circuit potential (OCP)—The evolution of the OCP as a function of time differed broadly depending on the media (Figure 5). The potential of the alloy in the presence of control medium and the medium collected after 24 h of cell culture under normal conditions ranged from -0.4 V to -0.5 V vs. SCE. However, the OCP measured in media collected after 24 h incubation of cells (macrophages, monocytes, and osteoblasts) under inflammatory conditions was reduced, ranging from -0.7V to -0.8V vs. SCE. There was no statistical significance between the challenged media groups (osteoblasts vs. macrophages $p=0.265$, osteoblasts vs. monocytes $p=0.55$, macrophages vs. monocytes $p=0.06$). However compared to control medium and conditioned medium, challenged macrophage medium showed a significant reduction in OCP value ($p < 0.05$ and $p < 0.008$ respectively). No significance was observed with monocytes medium and osteoblasts medium in comparison to control and conditioned media (control vs. challenged monocytes, $p = 0.06$ and challenged vs. osteoblasts $p=0.421$; conditioned medium vs. challenged monocytes, $p=0.06$ and conditioned medium vs. challenged osteoblasts $p=0.33$).

3.3.3. Electrochemical impedance spectroscopy (EIS)—Representative Nyquist (real part of impedance (Z_{real}) vs. imaginary part of impedance (Z_{img})) and Bode plot (Impedance modulus (IZI value and phase angle vs. frequency) obtained from the EIS data for CoCrMo alloy under different media conditions are shown in Figure 6a & b. The Nyquist plots revealed a higher impedance for all the control media conditions studied. Whereas, for the media from different challenged cell cultures (macrophages, monocytes, and osteoblasts), the impedance values of CoCrMo alloy were lower, with macrophages challenged medium showing the lowest value. Challenged monocytes medium also provided

a lower impedance value compared to control medium, but higher than osteoblasts and macrophages. In addition, medium with H_2O_2 also provided lower impedance compared to control media studied, suggesting high corrosion kinetics of CoCrMo alloy in the presence of an inflammatory medium. The Bode plot also revealed consistent results with low phase angles and impedance vs. frequency values with challenged macrophages medium (Figure 6b).

A modified Randle's equivalent circuit was used to model the EIS data, to determine polarization resistances (R_p) and capacitance (C) of the electrochemical double layer at metal-solution interface with different media conditions were analyzed (Figure 6 c&d). The elements of the electrical circuit were R_p , and Q ($Q=Cdl^{-n}$ and $Q=C$, if $n>0.9$) are in parallel, and in series with solution resistance (R_s) between the working electrode (WE) and the reference electrode (RE). The ANOVA for the comparison of R_p between different test media was significant ($p=0.002$). Resistance to polarization (R_p) in the presence of all the media conditions except challenged osteoblasts and macrophages was approximately $6 \times 10^6 \Omega \cdot cm^2$. However, the challenged macrophages and osteoblasts media decreased the R_p significantly compared to the control medium with values of $1 \times 10^4 \Omega \cdot cm^2$ ($p=0.02$) and $4 \times 10^4 \Omega \cdot cm^2$ ($p=0.021$), respectively. Such low R_p values indicated higher corrosion kinetics in the presence of medium from challenged macrophages and osteoblasts than other media conditions studied. The ANOVA for the comparison of the capacitance was also highly significant ($p<0.001$). Pairwise comparison also exhibited higher capacitance for medium from macrophages ($3.5 \times 10^{-5} F \cdot cm^2$, $p=0.047$) compared to control medium.

3.3.4. Potentiodynamic studies—Representative potentiodynamic curves for all the electrolyte conditions are shown in Figure 7a. It was evident that the curves for different control media conditions were shifted towards lower current densities compared to inflammatory media conditions. The corrosion potential (E_{corr}) and evolution of the corrosion current density (I_{corr}) values were obtained by Tafel's estimation from potentiodynamic curves (Figure 7b &c). The Kruskal-Wallis test for the comparison of the corrosion potential between different test media was significant ($p=0.007$). The E_{corr} value was higher ($p=0.046$) with media containing H_2O_2 (0.151 ± 0.009 V vs. SCE) compared to the control medium, which was also in agreement with the OCP measurements. No significant difference was observed in the E_{corr} values from challenged media conditions of macrophages, monocytes, and osteoblasts with that of the control medium. The ANOVA of the comparison of the corrosion current density was also significant ($p=0.001$). In contrast to E_{corr} , I_{corr} values were significantly ($p<0.008$) higher with challenged macrophages ($2.18 \times 10^{-6} \pm 8.7 \times 10^{-7} A \cdot cm^2$) compared to control medium and all other conditions ($p<0.05$).

4.0. Discussion

Fretting and corrosion processes within THR modular junctions are complex and multifactorial^{22,23}. It was the goal of this study to generate *in vitro* experimental evidence for cell-accelerated corrosion. The results support our hypothesis that macrophages challenged with wear particles can generate an environment that negatively impacts the

corrosion kinetics and corrosion rate of CoCrMo alloy. Thus, it appears possible that macrophages could contribute to corrosion processes occurring in THR modular junctions.

Our results have shown that monocytes, macrophages, and osteoblasts could be challenged with wear particles generated from CoCrMo alloy at 1 g/ml concentration with an average cell viability of > 88% after 24 h of incubation. The resulting media, after 24 h of culture, was then used as an electrolyte to test the corrosion kinetics and corrosion rate of CoCrMo alloy using standard electrochemical methods. This approach has several limitations. First, wear particles used to simulate inflammatory conditions were generated under sliding wear. This approach was chosen because wear particles can be generated at a higher rate, making it likely that the majority of particles are released into the wear testing solution, whereas particles from fretting tests are more likely to form films and agglomerates of oxide particles that stick to the sample surfaces and therefore are more difficult to isolate⁹. However, wear particles generated under sliding wear may differ from wear debris and corrosion products generated under fretting-corrosion within modular junctions. To date, there are no studies that can accurately describe the size and composition of fretting particles generated within modular junctions^{24,25}. Most studies focus on larger flakes that can be removed from the taper surfaces^{8,24}, yet the majority of particles are likely much smaller. It is also likely that such particles dwell in the crevice and form thick deposit layers. Such layers consist predominantly of chromium-oxide, CoCr particles, and metallo-organic complexes^{8,9}. Most cases with ALTRs also exhibit chromium phosphate particles, but the conditions which form these particles are unknown. The type of *in vivo* fretting particles that is generated strongly depends on the fretting regime (partial slip or gross slip)²⁶ and the underlying mechanism (abrasion, surface fatigue or tribochemical reactions)²⁷. So far there is no consensus on which fretting regime is predominant, although retrieval findings suggest that at least initially, partial slip occurs⁹. But the fretting regime and wear mechanism may change over time. The resulting particle composition may further differ due to the transformation from the moment of initial generation to the moment of phagocytosis based on particle size and chemical environment^{28,29}. The particles generated here appear to contain both CoCrMo alloy and chromium-oxide particles. As particles were generated within the bovine serum, metal-protein complexes will likely be present as well. Thus, particles generated in this study will at least partially resemble those generated under fretting within the modular junction. Another limitation of this study is that only macrophages, monocytes, and osteoblasts were considered to represent periprosthetic cell types, but other cell types, such as osteoclasts and fibroblasts may also have a role.

4.1 Generation of a corrosive environment by macrophages

Activation of cells by the addition of CoCrMo alloy wear particles clearly resulted in changes of the corrosion kinetics of CoCrMo within the conditioned media. In terms of different cell types, the corrosion tendency was noticeably higher in media from challenged macrophages and osteoblasts (compared to monocytes), and was significantly higher when compared to control media. Similarly, EIS and potentiodynamic results further confirmed that the cell medium from challenged macrophages negatively influences the corrosion rate and kinetics of CoCrMo alloy compared to other cell culture conditions. The results of this study demonstrate that under inflammatory conditions, macrophages can alter the chemical

environment to promote corrosion and compromise the corrosion behavior of CoCrMo alloy. The effect of other cell types was negligible, although medium from challenged osteoblasts did result in a lower polarization resistance and higher capacitance compared to control media.

These results confirm the model of cell-accelerated corrosion proposed by Gilbert et al., suggesting that macrophages release reactive oxygen species under inflammatory conditions and thus promote corrosion^{14,30,31}. One of the potential reactive oxygen species occurring *in vivo* is H₂O₂. In this study, we have used 0.03M H₂O₂ containing medium as a positive control for reactive oxygen species. Consistent with a previous report²¹, the corrosion tendency (OCP and E_{corr}) of CoCrMo alloy in the presence of medium with 0.03M H₂O₂ was significantly higher (towards noble potential), compared to other media conditions. This effect could be attributed to the changes in the passivation kinetics in the presence of H₂O₂, potentially protecting the CoCrMo metal surface²¹. However, this effect was not observed in medium from challenged macrophages. It is, therefore, possible that H₂O₂ is not the primary acting reactive oxygen species responsible for the observed corrosion kinetics. For example, macrophages could release inflammatory cytokines, chemokines, inflammatory enzymes, or proteolytic enzymes such as matrix metalloproteinases. Hence, the practice of H₂O₂ containing solutions as a positive control for such experiments might need additional consideration. It is important to note that media from challenged macrophages had the lowest pH. However, a pH of 6.74±0.14 is not low enough to explain the significant shift in OCP, R_p, C_p, and I_{corr} that was observed here or the severe corrosion features observed on retrieved implants⁹. It is more likely that metal wear debris activates macrophages through a unique cellular mechanism to generate a corrosive environment. The exact combination of the different reactive oxygen species and other chemical species secreted by macrophages is unknown and needs to be determined in detail. However, the results of our study demonstrate that the release of this combination of chemical species is able to compromise the corrosion behavior of CoCrMo alloy within the confined microenvironment of THR modular junctions. One of the limitations in the evaluation of potentiodynamic data is the subjective nature of the Tafel's estimation. Therefore, experiments were conducted in triplicates, and the mean values are reported. The fairly large deviation of the results is typical for this evaluation method, yet significant differences in E_{corr} and I_{corr} could be detected. Also, it is important to note that all the electrochemical experiments were conducted under atmospheric conditions, not in CO₂-enriched atmosphere. Therefore, a slight variation in pH can occur as reported in a previous study³², but this pH change is inconsequential for the corrosion behavior of CoCrMo, and all tests were conducted under the same conditions.

4.2 Model of cell-accelerated corrosion

In a previous study, cell-like features were found on the head taper surfaces of some femoral heads⁹. These features were clearly separate from one another, had a size range from 20 to 50 micrometers, and had a comparable morphology to that of cells such as macrophages in a scanning electron microscope (SEM). In an earlier study, some surface damage features on retrieved implant surfaces—in areas outside of modular interfaces—have been attributed to cell-induced corrosion¹⁴, but most of these features were later explained to be caused by the

use of electrocautery during revision surgery^{33,34}. In contrast, the previously described cell-like features are located within the contact zone of the head/stem taper surfaces. Specifically, these features were usually seated within areas of column damage. Column damage is characterized by long etched troughs running along the taper axis^{9,35}, and is associated with banding of the CoCrMo alloy microstructure³⁶. Considering our findings in this study, it is probable that fretting within modular taper junctions will eventually release particles from the crevice. Such particles may attract monocytes and macrophages to the crevice. Under optimal head-neck seating conditions, the interface is too tight to allow fluid to pass into the crevice formed by the modular junction interface. However, if the femoral head is not seated properly, or if the taper surfaces engage proximally in the head taper because of the taper design, an opening could occur and cells could gain access to the crevice. It is also possible that ongoing fretting processes may lead to a local widening of the crevice. Macrophages that possibly enter the crevice will be exposed to particles and particle agglomerates generated during fretting. Activated cells may be able to adhere to the taper surfaces and continuously release reactive oxygen species and cytokines under their cell membrane (cell-metal interface) resulting in eventual chemical changes of the microenvironment of the metal surfaces. Depending on the number of cells and considering the confined environment of the crevice, the concentration of chemically active species may be high enough to detrimentally change the corrosion kinetics on the CoCrMo alloy surfaces and increase the corrosion rate. The change of the crevice chemistry may explain harsh damage modes such as column damage, etching and pitting on the head taper surfaces, and intergranular corrosion phase boundary corrosion and pitting on the stem taper surfaces as earlier reported^{6,9,37,38}. These damage modes can contribute significantly to fretting-induced material loss. It is also likely that macrophages will undergo necrosis due to the high particle and metal ion burden. The release of cell contents into the crevice during necrosis may further contribute to the harsh chemical environment within the crevice. Ongoing fretting will continue to recruit more inflammatory cells to the site, which will, in turn, accelerate corrosion processes within the taper junction. The process continues synergistically in a cyclic manner, thereby assisting the occurrence of corrosion modes that eventually accelerate the damage modes, as illustrated in Figure 8.

Our previous retrieval findings and the electrochemical data of this study suggest that the contribution of macrophages to corrosion occurs as a result of fretting. Macrophages can only contribute to the corrosion process after fretting occurs, and a pathway for cells into the crevice is provided. Therefore, the term, cell-accelerated corrosion (CAC) may be more appropriate than cell-induced corrosion. The contribution of CAC to material loss cannot be easily quantified and may differ broadly. At least in the case of column damage, one can estimate that the contribution is significant as it covers large areas and leaves troughs with a depth of several tens of micrometers^{9,36}. Column damage is also inherently related to the alloy microstructure as are other damage modes such as phase boundary corrosion and intergranular corrosion^{35,37,38}. Thus, the potential impact of CAC is also dictated by the alloy microstructure.

Although the results of this study support the theory that CAC may be an important contributor to corrosion processes within THR modular junctions, further studies are required to determine the transitions in the electrochemical mechanisms/kinetics and overall

impact on the material loss. Therefore, a detailed evaluation of molecular pathways and cellular phenotypic differences in macrophage cells during corrosion are the future perspectives of this study. In addition, the study utilized low carbon CoCrMo alloy instead of high carbon CoCrMo alloy for all the electrochemical tests, which will be considered in future evaluations.

5.0. Conclusions

In conclusion, this study has successfully established an *in vitro* test to characterize the corrosion behavior of CoCrMo alloy in conditioned cell culture media of different periprosthetic cell types under normal and inflammatory conditions. The results demonstrated that macrophages had the highest particle uptake and had a significant effect on the corrosion rate as measured by I_{corr} and the overall corrosion kinetics as measured by the OCP, R_p , and the Nyquist plots. Thus, macrophages can alter the chemical environment in the presence of wear debris and compromise the corrosion behavior of CoCrMo alloy. These results and earlier retrieval findings suggest the following *in vivo* model: fretting wear and corrosion induced by micromotion may release wear debris that recruits monocytes/macrophages to the site of the modular junction. If the crevice is wide enough, macrophages may enter the crevice and change the chemical environment, give rise to chemically dominated damage modes, and subsequently accelerate material loss. Further studies are needed to determine the exact mechanisms of CAC and its overall impact on metal degradation and metal ion release. A detailed evaluation of molecular pathways and cellular phenotypic differences in macrophages during corrosion needs to be further investigated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Schematic representation of the overall study outline.

Figure 2:

a) Schematic representation of the tribocorrosion hip-simulator and b) corrosion apparatus connected to the potentiostat through a three-electrode system, where the CoCrMo disc is the working electrode fully immersed in the electrolyte.

Figure 3:

a) DLS spectrum showing the particle size range of CoCrMo wear debris generated from the hip simulator. b) Scanning electron microscopic image of wear debris. c) Results of the EDS analysis of CoCrMo wear debris to confirm the presence of cobalt and chromium. The results are qualitatively due to the fact that lighter elements such as carbon and oxygen cannot be accurately quantified by this EDS system¹⁹. However, there appeared to be a fairly large amount of oxygen indicating the presence of metal oxides, likely chromium oxide. The overall dominant presence of cobalt compared to chromium indicates that the majority of debris is still cobalt-based alloy. The presence of carbon, sodium, and chlorine indicates the presence of organic residue and salt from the wear testing solution.

Figure 4:

a) Graph showing cytotoxicity of wear particles to osteoblasts, macrophages, and monocytes at different concentrations after 24 h. Data are presented as Mean $\pm$ SD. b) Dot plot obtained from particle uptake by osteoblasts, macrophages, and monocytes after 24 h incubation with wear debris (concentration: 1g/ml) using flow cytometry compared to -ve control (cells without particle treatment). c) Corresponding graph showing side scatter events by the cells. Data are presented as Mean $\pm$ SD. * $p < 0.05$. N=3 and all experiments were repeated three times.

Figure 5:

OCP of CoCrMo alloy samples within different media. H₂O₂ had the highest OCP, whereas media from cells cultured under inflammatory conditions had the lowest OCP. N=3 and the experiments were repeated three times.

Figure 6:

a) Nyquist and b) Bode plot of CoCrMo alloy based on EIS data from CoCrMo alloy tested within different media. (c) Resistance to polarization (R_p) and (d) capacitance (C_{dl}) of CoCrMo alloy within different media. N=3 and all experiments were repeated three times.

Figure 7:

a) Potentiodynamic polarization curves of CoCrMo alloy within the different cell culture media and control electrolytes. Bar graphs showing b) E_{corr} and c) I_{corr} values of CoCrMo

alloy within different cell culture media and control electrolytes. N=3 and all experiments were repeated three times.

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Figure 8:

Schematic representation of the pathway of cell assisted corrosion (CAC) within the neck tapered modular junction of THRs. Wear particles released due to the micromotion of the head and neck modular junction attract inflammatory cells, including monocytes and macrophages, to the site. Wear debris causes polarization of monocytes to specific macrophage lineage (phenotype is unknown) and activate macrophages at the head-neck interface. These activated cells will secrete factors (ROS, cytokines, chemokines, and other unknown factors), which cause a corrosive environment at the cell-material interface. This may lead to a cascade of events which changes the overall chemical nature of the microenvironment within the crevice, further accelerating the corrosion damage.

pH values from different media after incubation (media without cells) or 24h cell culture (medium with cells) and before electrochemical analysis. The media includes control, medium with DP, conditioned medium, and challenged medium from macrophages, monocytes, and osteoblasts.

Table 1:

Samples	Control Medium (G1)		Medium with DP(G2)		Medium with H ₂ O ₂ (G3)		Conditioned Medium(G4)		Challenged Medium Treated with 1 µg/ml of DP			
	DMEM RPMI		DMEM RPMI		DMEM RPMI		DMEM RPMI		24 h			
									Macrophages (G5)	Monocytes (G6)	Osteoblasts (G7)	
	DMEM medium, stored in the fridge and warmed to 37°C		Medium, stored in the fridge, warmed to 37°C and treated with 1 µg/ml of DP		Medium stored in the fridge and warmed to 37°C and added 0.03M H ₂ O ₂							
N1	7.62	7.26	7.61	7.36	7.63	7.35	7.22	7.33	6.79	7.23	7.39	
N2	7.60	7.35	7.54	7.32	7.61	7.36	7.34	7.35	6.58	7.31	7.52	
N3	7.59	7.31	7.59	7.31	7.64	7.35	7.35	7.39	6.87	7.21	7.35	
AVG	7.60	7.34	7.58	7.33	7.62	7.35	7.30	7.35	6.74	7.25	7.42	
STDEV	0.01	0.02	0.03	0.02	0.01	0.005	0.07	0.03	0.14	0.05	0.08	

AVG-Average, STDEV-Standard Deviation, G1-G7- Group1-Group7