



# SMART Biosensor for Early Diagnostic Detection of Metal Ion Release in Orthopedic Patients: Initial Outcome

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## Abstract

Metal ion release from the orthopedic implants is a great concern for the clinicians and patients. Currently, the inductive coupled plasma-mass spectroscopy (ICP-MS) technique is used to detect and estimate the metal ions in the blood or synovial fluid, which is expensive and needs technical assistance. Hence, the aim of the current work is to develop a biosensor based on the electrochemistry to measure the metal release to the body fluids (blood or synovial fluid) from the implants. This will work very similar to a glucometer (by function), as a patient-driven technique, if it is optimized for the blood samples, and for clinical purpose, in case of the synovial fluid estimation. As a proof of concept effort, the present study has two objectives: (1) To study the effectiveness of using a micro-chip biosensor as a diagnostic technique for the early detection of the released metal particles in the synovial fluid solution (study 1), and (2) to investigate the corrosion kinetics of CoCrMo alloy in the presence of metal particles in synovial fluid solution (study 2). A series of tests were done with biosensor prototype with increasing concentration of metal release (particles and ions), which is generated from a hip simulator (study 1). The impedance variation ( $\Delta Z$ ) shows a very close correlation with increased amount of metal release (particles and ions) level (study 2). Although the study has several limitations, the initial findings indicate that a biosensor could be developed as a diagnostic tool to detect the metal release (particles and ions) levels.

**Keywords** Impedance · Biosensor · Metal ion/debris · Hip implants

## 1 Introduction

The use of biomaterials in vivo is becoming increasingly common among many fields of medicine. In the field of orthopedics, specifically, metal implants are commonly used in total joint arthroplasty. Because of this, total hip replacements (THR) remain one of the leading procedures regarding success rates and research advancement in Orthopedics. However, complications related to the implant metal composition and implant longevity are still severely limited

by tribocorrosion and its effects leading to implant failure [1–3].

Tribocorrosion is the interacting processes of mechanical wear and chemical corrosion on the implant [4–6]. The exact mechanism remains unclear, but it is generally accepted that mechanical wear removes the protective passive oxide layer on the implant exposing the underlying alloy to electrochemical reactions, leading to corrosion [7, 8]. Studies have shown that the interaction between wear and corrosion leads to a synergistic increase in metal debris and ions production [4]. The release of metal particles induces an inflammatory reaction known as adverse local tissue reaction (ALTR) and pseudotumor formation [9–11], which has been commonly reported as a reason for revision surgery. Correspondingly, there has been an association noted between metal release (particles and ions) and revision rates [12, 13]. Additionally, elevated serum metal ions have been associated with increased carcinogenesis, infection, immune suppression, hypersensitivity reactions, and progressive cardiomyopathy [14–17].

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Currently, serum metal ion levels in patients are analyzed using inductively coupled plasma mass spectroscopy (ICP-MS) [18, 19], which is a highly sensitive spectroscopic technique that is versatile in simultaneous determination of heavy metal ion concentrations. The downfall to this technique is that it is very expensive and not widely available, as it requires trained personnel to work complex equipment and requires multi-sample preparation [20]. This has restricted metal ion surveillance to annual occurrences or when the patient presents symptoms that relate to pain or possible prosthesis complications. In many instances, there is poor follow-up with patients that can foster long periods of time for corrosion to occur with systemic exposure to toxic levels of metals. Toxic levels of metal are considered to be highly hazardous to the human health even at trace amounts. Therefore, a fast, sensitive, and economic method for determining metal ions could permit better management and deterrence of complications related to metal implants. Current concepts have employed electrical impedance spectroscopy (EIS) for detection of trace levels of pesticides in ground water as well as biomarkers from low-volume body fluids [21]. Augmenting this application to the detection of Co, Cr, and Mo ions can provide an affordable and accessible alternative to ICP-MS for metal ion surveillance.

A screen-printed electrodes (SPEs) can meet this low cost expectation as well as the requirements of analytical sensors in terms of sensitivity, selectivity, stability, precision, and ease of use [22]. SPEs have a flexible design and total automatization in the fabrication of a complete system that contains a working, counter, and reference electrode.

Utilizing this technology alongside a metal ion electrochemical biosensor (MIEB) with their ease of preparation and design, SPEs possess an advantage over other devices [23–26].

The present study has two main objectives: (1) To study the effectiveness of using a micro-chip biosensor as a diagnostic technique for early detection of the released metal particles in the synovial fluid solution, and (2) to investigate the corrosion kinetics of CoCrMo alloy in the presence of metal particles in synovial fluid solution.

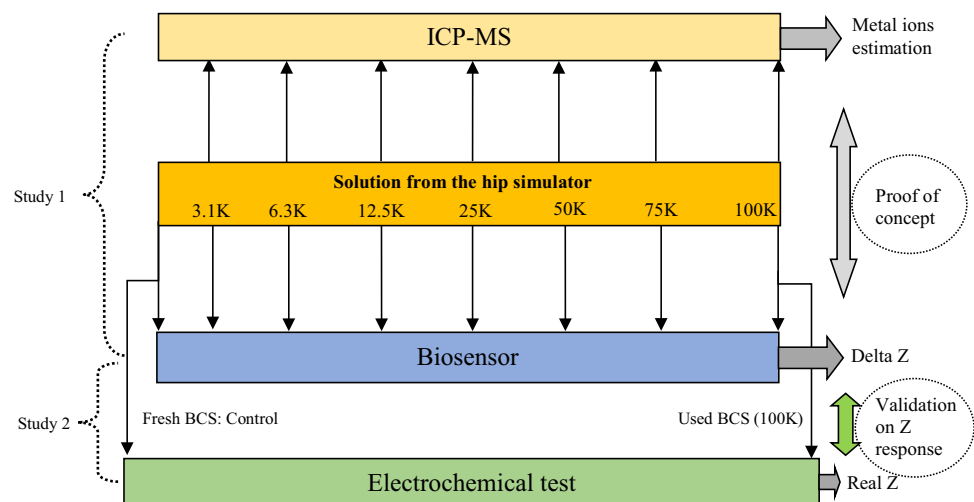
## 2 Materials and Methods

A schematic diagram of the study design is provided in Fig. 1. Study 1: proof of concept study to understand the trend of impedance variation and metal release (particle and ions) as a function of cycles up to 100K. Study 2: To validate the impedance response from the biosensor with impedance resulted from the basic electrochemical test, by exposing to CoCrMo alloy. More details are included below.

### 2.1 Metal Release (Particles and Ions) Collection

A custom-made hip simulator is used in this study, which consists of a CoCrMo pin (composition is provided in Table 1), is in contact with a ceramic ball immersed in the bovine calf serum (30 g/L). The BCS solution contained BCS (30 g/L protein content), NaCl (18 g/L), Propylene Phenoxenol (30 mL/L), Tris (54 g/L), and HCl (approximately

**Fig. 1** Study design: Schematic diagram of the study design; Study 1: proof of concept study to understand the trend of impedance variation and metal release (particle and ions) as a function of cycles up to 100K. Study 2: To validate the impedance response from the biosensor with impedance resulted from the basic electrochemical test, by exposing to CoCrMo alloy



**Table 1** Chemical composition (in weight percent) of the CoCrMo alloy

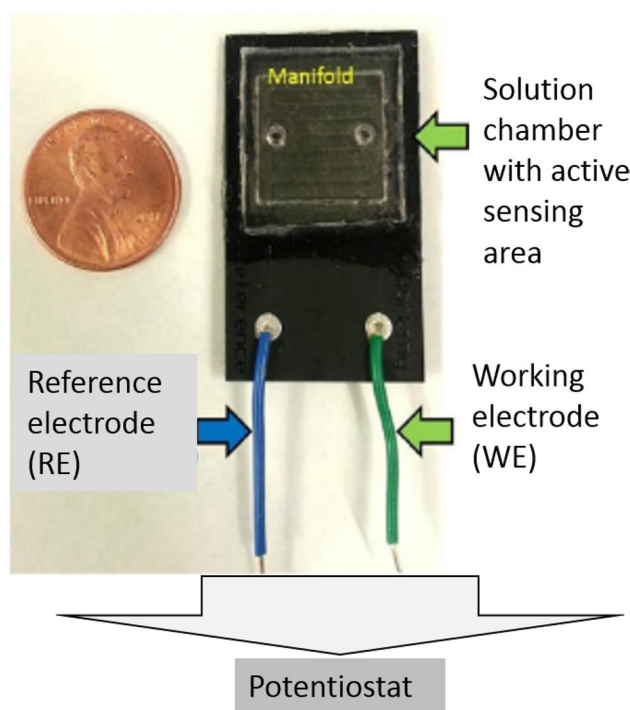
CoCrMo alloy	Composition (in wt%)						
	Co	Cr	Mo	C	Si	Mn	Al
	64.60	27.63	6.04	0.241	0.66	0.70	0.02

55 mL/L, or enough to bring the pH of the solution down to 7.6). The ball was rotating at ± 30° of rotation with a frequency of 1 Hz and a constant load of 16N (approximately 50 MPa: initial contact pressure) was applied. The experiment runs for a period of 100K cycles, the solution samples (1.5 ml) were collected periodically (3.1K, 6.3K, 12.5K, 25K, 50K, 75K, and 100K) and replenished with fresh solution (see Fig. 1). The metal ion content was estimated by ICP-MS techniques, as shown in Fig. 2. The trend in the metal ion release as a function of cycles will provide a baseline data to interpret the biosensor response.

### 2.2 Biosensor Tests

The chip was designed similarly to a three-electrode system with the working principle based on electrochemistry. A picture of the existing printed circuit board design of the biosensor is provided in Fig. 3. The assembled device has a polymer manifold to contain fluid on the gold sensing site. The sensor uses 100 µl of sample volume per analysis. The test methodology was based on the hypothesis that, with increasing sliding durations, there should be an increase in metal release (particles and ions) concentration. This would, in turn, result in a decrease in impedance (increased corrosion of implant) measured through EIS for each consecutively used BCS sample. It was designed in a similar construct to a standard 3-electrode electrochemical system.

The biosensor measured the impedance of the solution applied, which is differing concentrations of metal release. Impedance is a vector measure of resistance, in the case of AC voltage applied in EIS test. More specifically, it allows an adequate description of the alloy’s resistance to corrosion. Change of impedance and percent change of impedance were both measured using the following equations:



**Fig. 3** A picture of Metal-Ion Electrochemical Biosensor (MIEB) working principle is Electrochemical Impedance, total size can contain on a palm (4 cm × 2.1 cm). The solution will be applied to the chamber. The working electrode (WE) and Reference electrode (RE) will be connected to the Gamry made potentiostat

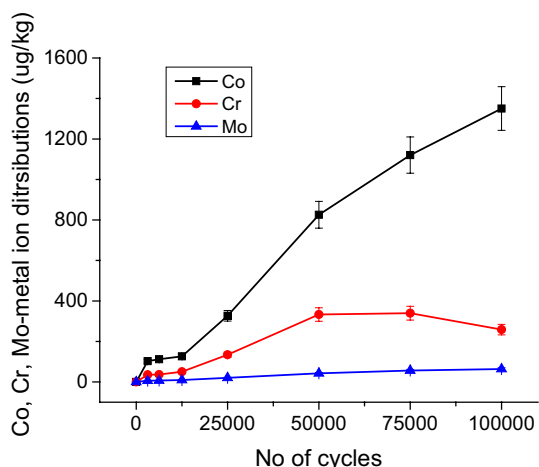
$$\Delta Z = Z_0 - Z_f$$

$$\% \Delta Z = (\Delta Z / Z_0) \times 100,$$

where  $Z_0$  is the initial impedance [control with metal release (particles and ions)] and  $Z_f$  is the final impedance (differing concentrations of metal release). Once the impedances were calculated with the biosensor, these values were compared with those obtained with standard electrochemical techniques to demonstrate the reliability of the biosensor data. The concentrations of metal release (particles and ions) as increasing the sliding cycles (total seven intervals) used such as control (fresh solution), 3.1K, 6.3K, 12.5K, 25K, 50K, 75K, and 100K were evaluated (as shown in the metal ion release data in Fig. 2).

### 2.3 Electrochemical Test

In this study (study 2), to validate the impedance response from the biosensor, an additional standard electrochemical tests were conducted with collected solution at the end of 100K cycles. In fact, tests were conducted on CoCrMo alloy samples with two solutions: BCS solution with high metal content (100K solution referred to as “Used BCS”) to simulate synovial fluid after wear and pure BCS as a



**Fig. 2** Metal ion distribution—Tribocorrosion test using in vitro hip simulator—CoCrMo—Potentiostatic conditions

control (“Control BCS”). A standard electrochemical cell of three electrodes was designed similarly to the biosensor. The counter electrode (CE) was graphite, the reference electrode (RE) was saturated calomel electrode, and the working electrode (WE) was the CoCrMo alloy disc. The CoCrMo samples were high carbon discs 12 mm in diameter and kept the surface roughness below Ra value of 20 nm. The exposed area to solution of 0.38 cm<sup>2</sup>. Both basic corrosion tests and standard impedance testing were run per sample via a potentiostat (Gamry Instruments, PA USA). In each experiment (*N* = 3), the following sequence of tests were conducted: Open circuit potential (OCP), potentiostat (PS), OCP, electrochemical impedance spectroscopy (EIS), cyclic polarization, and OCP with potential at *E*<sub>corr</sub>. A Warburg circuit model was used in calculating impedance parameters. Corrosion Potential (*E*<sub>corr</sub>), Corrosion rate (*I*<sub>corr</sub>), and Total Impedance (*Z*) were estimated.

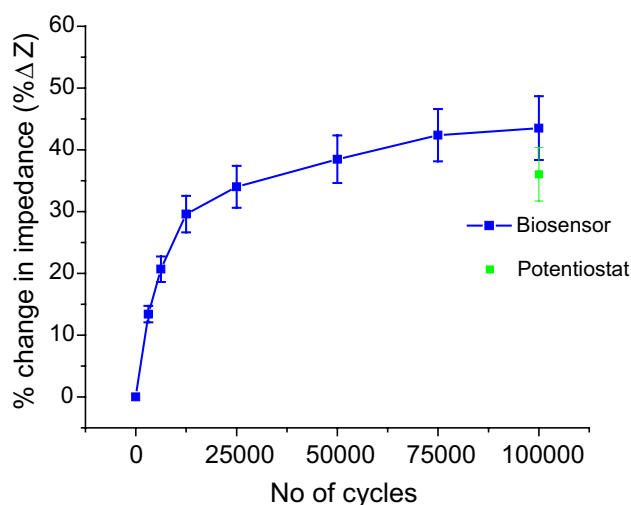
Both the biosensor and corrosion kinetics tests were run in similar conditions maintained at 37 °C with a pH of 7.6.

### 3 Results

The impedance measurements (*n* = 3) by the biosensor as a function sliding cycles in list in Table 2 and displayed in Fig. 4. Generally, as the metal content increases, there is a decrease in the impedance values. By keeping the Control BCS as baseline, change in impedance was estimated ( $\Delta Z$ ) and the percentage change in impedance was determined (% $\Delta Z$ ). Even though there is an increasing % $\Delta Z$ , there is a sudden increase in the initial range of cycles which then exhibits a slower increment after 12,500 cycles. Additionally, it is demonstrated that there is no statistically significant difference between the standard potentiostat impedance measurement and the biosensor impedance measurement. *Z*<sub>t</sub> (Used BCS) = 5750 ± 575 Ohms and *Z*<sub>t</sub> (Control BCS) = 7400 ± 740 Ohms with no statistically

**Table 2** Biosensor responses: impedance values, mean *Z*, delta *Z*, and % change in *Z*, as a function of increasing metal release (particles and ions)

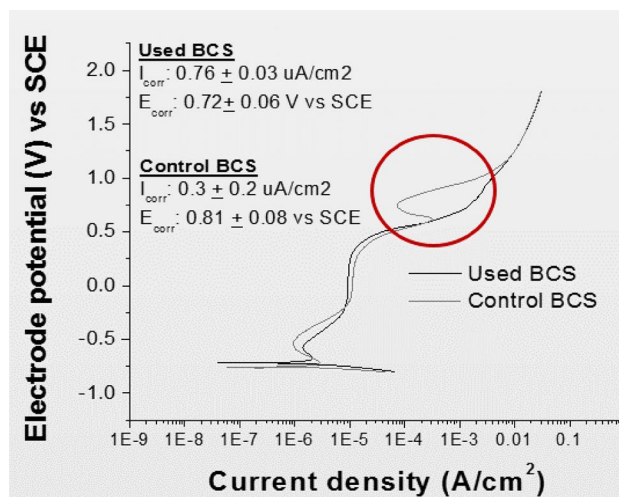
Sample	Mean <i>Z</i> (ohms)	$\Delta Z$ (ohms)	% Change
Buffer	4419.62		
Baseline (pure BCS)	4097.90	0.00	0.00
3.1K	2985.53	517.44	13.41
6.3K	2728.20	774.77	20.69
12.5K	2399.32	1103.65	29.53
25K	2247.40	1255.56	34.02
50K	2089.42	1413.54	38.47
75K	1964.60	1538.36	42.36
100K	1939.44	1563.53	43.51



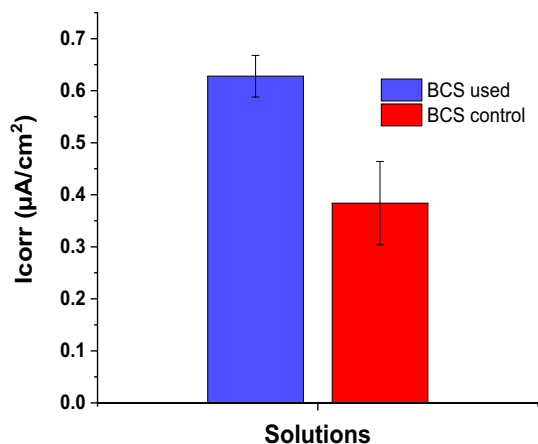
**Fig. 4** Percentage change in impedance recorded by biosensor in Study 1. The final impedance recorded by the standard EIS test with Used BCS solution (study 2). Both data, Biosensor and potentiostat are in acceptable range

significant difference between the two methods of impedance measurement—*p* < 0.003.

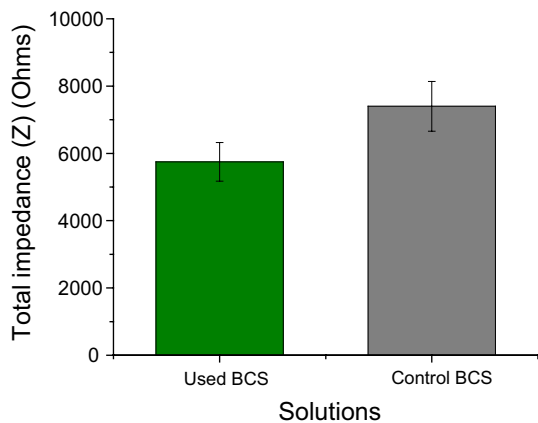
As mentioned earlier, the electrochemical results from the corrosion tests (study 2) are presented in Figs. 5 and 6. The potentiodynamic curves (Fig. 5) for Used BCS and Control BCS exhibit considerable differences. There is an increase in the corrosion behavior of CoCrMo alloy in Used BCS, demonstrated by high *I*<sub>corr</sub> values (rate of corrosion), and low *E*<sub>corr</sub> values compared to Control BCS (Fig. 7). Interestingly, for the Used BCS, the polarization data showed an absence of typical transpassive behavior (Fig. 5).



**Fig. 5** Potentiodynamic curves for Used BCS and Control BCS from electrochemical test from Study 2

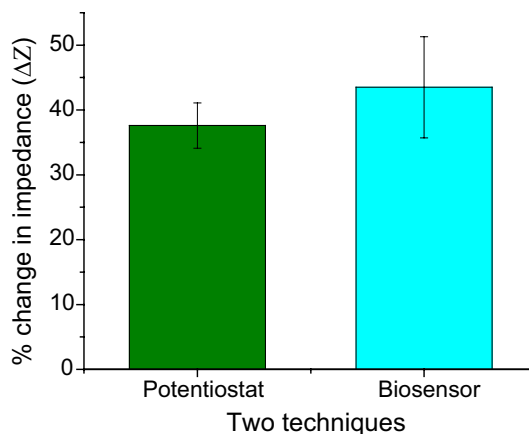


**Fig. 6** Corrosion current, ( $I_{corr}$ ), estimated through Tafel’s slope from the potentiostat from Study 2



**Fig. 7** Total impedance from EIS model for two tested solutions, Used BCS (with metal ions and particles) Control BCS (Fresh solution) from study 2

The impedance data were used to model an equivalent circuit and total impedance was calculated as shown in Fig. 6. In the presence of Used BCS, lower impedance (Z) was observed compared to control which demonstrates the increased corrosion kinetics (less resistance to corrosion demonstrating increased corrosion) of the CoCrMo alloy in the electrochemical process with Used BCS. The percent change in the impedance ( $\% \Delta Z$ ) of the CoCrMo sample with Used BCS was approximately 36.03% (Fig. 7). The  $\% \Delta Z$  obtained by using the biosensor for 100K cycles was a  $43.5\% \pm 7.8\%$  which compares well with the data received using the potentiostat ( $\% \Delta Z$ :  $36.03 \pm 3.5\%$ ), ( $p=0.4$ ). (see Fig. 8).



**Fig. 8** Percentage change in impedance at 100K cycles from biosensor and biosensor (Study 2). Both results show good agreement

### 4 Discussion

As implants are used, they release increasing amounts of metal release (particles and ions) into the synovial fluid, which can result in many local and system complications, such as various diseases and disorders to the nervous, reproductive, immune, and gastrointestinal systems. Even the smallest dose of these metal releases (particles and ions) can adversely impact human health. The results show that as the concentration of metal particles increase, there will be an increase in conductivity and a decrease in impedance. This decrease in impedance will enhance corrosion of the CoCrMo alloy; thus, the higher the concentration of metal release (particles and ions), the more corrosion of the metal alloy that will take place. The effect of decomposed form of protein could not be neglected. However, this study demonstrates that metal release (particles and ions) do increase corrosion. Understanding how implants are affected by the metal release (particles and ions) may guide the future care of patients both therapeutically and diagnostically; therefore, there is a need to develop a method for determining trace amounts of these metal release (particles and ions) in biological samples such as blood, serum, and saliva [9, 27]. Additionally, our preliminary studies indicate that a biosensor can be developed to accurately measure the presence of metal release (particles and ions); and thus, the extent of corrosion of an implant for the diagnostic purposes (Figs. 4, 8). Existing methods of spectroscopic and optical techniques for metal release detection are reliable, but do not yield the same economic and user-friendly approach as electrochemical techniques for such applications. The electrochemical results demonstrate that the presence of metal release (particles and ions) decreases impedance, increases rate of corrosion in Used BCS, and prevents passivation kinetics in the BCS solution. This is further supported by the  $I_{corr}$ , rate of

corrosion (Fig. 5), being significantly higher in the presence of the metal release (particles and ions). Lastly, the fact that there is lower impedance describes the material surface's ability to resist corrosion. Since there is less resistance to corrosion, an increase in corrosion kinetics will be demonstrated, and the implant will be put at risk of early failure. (Figs. 6, 7).

Thus, we demonstrate that metal release (particles and ions) in the environment of the implant will increase the corrosion kinetics of the implant. The applicability of metal-ion electrochemical biosensor as a quick, easy, and cost effective biosensor chip in detecting metal release (particles and ions) in solution is now established. An electrochemical technique offers a short analytical time, lower sensitivity to improve its performance in detecting metal release (particles and ions), and allows for a simple procedure that allows for *in situ* monitoring. Hence, biosensor could be considered as a potential diagnostic tool [28] for the orthopedic patients and clinicians to gauge the extent of corrosion of implants and for assessing future treatment options for the patient. The current study has many limitations, include the solution was directly from the hip simulator that contains metal ions and metal particles/debris and provide only a proof of concept and a baseline data for developing this valuable diagnostic tool for the orthopedic patients. Further studies will be considered to calibrate the biosensor and check its sensitivity to detect individual metal ions (Co, Cr, and Mo) distributions, particularly its specificity.

#### 4.1 Clinical Significance

Understanding the negative corrosive impact that these metal release (particles and ions) have on implant corrosion processes will guide future clinical treatment in extending implant longevity. Additionally, by using biosensors (MIEB), we can develop a diagnostic method of gauging the extent of corrosion to assist the orthopedic patients and surgeons to guide their management.

## 5 Conclusions

From this study, A biosensor prototype is developed specifically to measure the metal release (particles and ions) levels in the body fluid. The impedance variation with increasing metal release (particles and ions) indicated that the biosensor can be an effective diagnostic tool. The results of this proof of concept study provided preliminary data to develop the biosensor. Further research is required, including validation of the sensor and specificity of the responses to metal particles and metal ions.

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