Electrochemical Biosensor – a promising diagnostic tool to detect implant-generated wear particles.

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INTRODUCTION: Total hip replacement (THR), a significant surgical procedure intended to reduce pain and improve mobility in people suffering from conditions like osteoarthritis, rheumatoid arthritis, post-traumatic arthritis, avascular necrosis, and childhood hip disease, is performed on about 1.2 million patients annually in the US[1,2]. Tribocorrosion, material deterioration, infection, inflammation, and implant loosening are some of the major causes of THR failure[2]. Tribocorrosion of hip implants can produce wear particles such as Co ions, Ti ions, and CoCr particles, which ultimately contribute to both local and systemic toxicity. The recent studies reported that antioxidants including vitamin E, Eugenol, and Carvacrol function as corrosion inhibitors to combat this problem, significantly reducing the creation of wear particles [3,4]. However, only a few non-invasive techniques, including plasma-mass spectroscopy (ICP-MS), are available to detect and monitor the presence of these corrosion and/or wear products. Nevertheless, this method is pricey, and its application calls for highly qualified specialists[5]. In our group, we used an electrochemical biosensor, as solution for these concerns and provided a very interesting initial outcome. Therefore, we hypothesize that the creation of an affordable and convenient diagnostic tool is imperative for consistent monitoring of wear particles over a hip replacement's lifetime. To that end, the main objective of this project is to develop an electrochemical biosensor that can accurately detect these wear particles and the effect of antioxidants on them. The redox reactions caused by these wear particles may be translated into quantifiable electrochemical signals, such as capacitance, resistance, and impedance, using electrochemical biosensors[6].

METHODOLOGY: a) Synthesizing and Characterizing wear particles: The wear particles (CoCr particles, Co ions, and Ti ions) were procured from Bioengineering Solutions, Chicago, and characterized based on size and morphology using dynamic light scattering and SEM analysis respectively. b) Plasma collection: C3HeJ Mice (Sex: Male, Weight: 25g, Strain:000659) obtained from the Jackson Laboratory were divided into 6 groups based on the treatments which include wear particles (CoCr particles, Co ions, Ti ions) and CoCr particles followed by antioxidants (Vitamin E, Eugenol, and Carvacrol). All these wear particles (10µl of 50ppm) and antioxidants (10 µl of 2.5ppm) were injected into the intraarticular joint space of the mouse's right knee [IACUC ID:1684850-3]. The blood samples (500 µl to 1 ml) were collected by tail snip method on 14, 28, and 35 days. Following centrifugation (800 rpm for 5 min), the plasma was extracted. c)Detection of metal ions using Electrochemical biosensor: The extracted plasma was analyzed using Gold screen-printed Electrodes (DRP-250AT) using our lab-prescribed protocol. Firstly, Crosslinker (DSP) was added to ethanol cleaned biosensor to bind the analytes. Following that by adding 50 µl of plasma samples in 1:200 dilution, the biosensor was subjected to various electrochemical analyses such as electrochemical impedance spectroscopy (EIS) and Cyclic voltammetry (CV). The data was collected from Gamry interface 1000 potentiostat and analyzed using the CPE model (simplified Randles circuit) of Gamry software (Fig 1), based on which bode and Nyquist plots were constructed (Fig 2 a,d,e,f). d) SEM Analysis: The presence of the metal ions on the surface of the biosensor was further confirmed using SEM-EDAX analysis.

RESULTS: The treatment groups had a statistically significant increase in impedance compared to the negative control. This finding demonstrates the biosensor's capacity to quantify wear particles and indicates that electrochemical processes occur on its surface. Additionally, we noticed that the presence of wear particles caused an increase in capacitance and a decrease in resistance (Fig 2 c, d). The pattern obtained is similar to that of corrosion processes observed on the biosensor**.** On the other hand, we observed a rise in resistance and a decrease in capacitance in plasma samples treated with CoCr particles and antioxidants when compared to plasma samples containing CoCr particles (Fig 2 g, h). The above result suggests that the antioxidants could have reduced the corrosion effects of CoCr particles, which helped to change the electrochemical reactions. Through SEM-EDAX analysis, the presence of wear particles on the biosensor surface was further confirmed**.** GraphPad Prism Software was used for the statistical analysis, and the results were assessed using a Paired T test. The criterion for significance was set at $p< 0.05$**.**

DISCUSSION: The results of our study conclusively show that the newly developed electrochemical biosensor is capable of correctly recognizing wear particles coming from implants. The examination of key electrochemical characteristics, such as capacitance (C), resistance (Rp), and impedance (Z), is the foundation of this effective detection (Fig 3). Further, the addition of antioxidants to the system causes the creation of a shielding film around the implant. As was already mentioned[3,4], this protective layer considerably reduces the production of wear particles. Our diagnostic tool's impressive ability to identify and record this result is evidence of its sensitivity and accuracy. In essence, the created biosensor exhibits a remarkable degree of sensitivity, which makes it an integral tool for implant wear particle measurement and detection. However, the present study has limitations of performing with small sample sets treated with smaller animal groups.

SIGNIFICANCE: A very effective, affordable, user-friendly, and quick diagnostic instrument designed for the identification and monitoring of implant-generated wear particles is the Dropsens electrochemical biosensor. By using this cutting-edge biosensor on a regular basis, proactive monitoring of metal ions in patients with implants is made possible, reducing the risk of local and systemic toxicity. The adaptability of this diagnostic tool also extends to its potential use in evaluating the efficacy of cutting-edge implant materials and therapies, including antioxidants, intended to reduce the formation of implant-generated particles. As a result, the biosensor is a useful tool for assessing and improving such therapies. Although our preliminary results are encouraging, more thorough research is required before this diagnostic tool may be used in real-world settings. The capabilities of the biosensor must be improved and validated by ongoing research in order to make it more ready for clinical application.

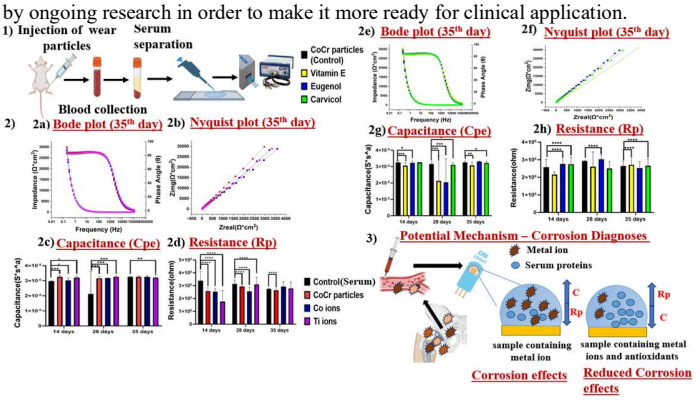

Figure 1: Represents the methodology of collecting the blood from the samples treated mice.

Figure 2: Electrochemical analyses: Depicts the Bode and Nyquist plots, capacitance, and resistance in the presence of wear particles (a, b, c, d) and antioxidants (e, f, g, h)

Figure 3: Diagnostic tool: Schematic diagram representing the ability of the developed biosensor to detect the presence of wear particles as changes in the electrochemical parameters with and without the presence of antioxidants.

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