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PREPARATION OF IN VITRO BIOIMPEDANCE TECHNOLOGY FOR MICRO- AND NANOPLASTIC TOXICITY STUDIES

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Abstract:
This work investigates the suitability of a custom-developed Bioimpedance Spectroscopy (BIS) sensor for the in vitro detection of micro- and nanoplastics, as well as the measurement conditions required to obtain reliable signal changes. The behavior of polystyrene-based particles across different concentrations, sizes, and surface coatings was explored. Experiments were conducted using in-house developed sensing plates and suspensions containing plastic fractions of various size ranges. The methodology included fabrication of the plates, validation with saline solutions, preparation of surface coatings, and repeated BIS measurements. Based on the results, particle detectability was characterized, and the effects of surface coatings on adhesion and washability were assessed. These findings provide a foundation for the development of standardized in vitro platforms and for future toxicological applications.

Keywords: *Engineering, In vitro assay, Toxicity Studies, Micro- and nanoplastic.*

Introduction

Since the 1950s, an increasing reliance on the industrial production of plastics has been observed, primarily due to low production costs, versatility, and high stability of polymers [1]. Plastic-derived waste is generated from various sources, including terrestrial disposal, wastewater treatment, textile shedding, and tire wear. Under the influence of environmental factors such as wind and UV radiation, polymer-based particles undergo physical and chemical degradation,

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leading to the destabilization of ecosystems. Furthermore, plastics are capable of accumulating pollutants and additives, which are potentially toxic [1]. It has been stated that dispersed micro- and nanoplastics can induce toxicological, physiological, and behavioral changes in living organisms, posing serious ecological and health risks [2]. Consequently, the investigation of the potential toxicity of these particles is considered an essential research task.

Particles smaller than 5 mm are referred to as microplastics [3], while those smaller than 1 µm are classified as nanoplastics. These particles exhibit diverse properties and originate from primary or secondary sources [4]. Due to partial scientific knowledge, the advancement of further research is required.

Bioimpedance spectroscopy (BIS) is applied as a non-destructive, real-time material analysis method [5]. In previous studies, the accumulation of 0,8–1 µm microbeads were induced in HepG2 human hepatoma cells [6]. Internalization was confirmed via fluorescence microscopy and flow cytometry. It was determined that the presence of the beads caused significant changes in the resulting BIS data [6].

Building on these preliminaries, the sensitivity of the *in vitro* bioimpedance technology is investigated. The method is evaluated for detecting micro- and nanoplastics of various sizes (0,7 µm and 15,5 µm) on self-designed measuring plates with different surface coatings (Fibronectin, Poly-D-lysine) [5]. The aim of the study is to determine whether the deviations detected by BIS originate exclusively from intracellular beads or whether particles remaining in the suspension are also detected [6]. The long-term goal is to provide a basis for *in vitro* human models applicable to future toxicological screening and health risk assessments.

1. The custom-designed BIS measurement technique

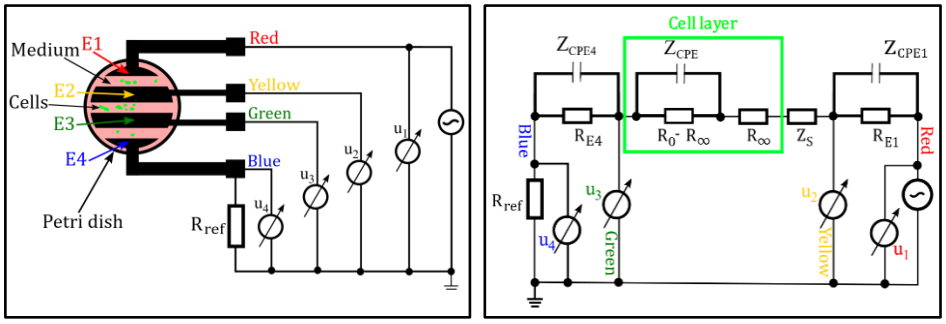
Bioimpedance spectroscopy (BIS) measurements were conducted using a self-developed, modified four-electrode arrangement, presented in

Picture 1., [5]. Through this technique, errors caused by excitation electrodes and residual impedances are simultaneously suppressed, significantly reducing the impact of contact and residual impedance. Since residual impedance distorts measured values, its compensation is considered essential for achieving accurate results.

In this system, the signal generator is connected directly to one electrode, while the grounding point is linked to the other through a resistor. Through this arrangement, potentials are shifted by a constant value, which is then compensated digitally during the impedance calculation. Digital processing removes common errors in symmetrical circuits through mathematical division and correction.

As illustrated in the arrangement in Picture 1., a constant voltage shift is created by a reference resistor (R_{ref}) connected in series with the measuring cell. Consequently, the excitation electrodes (E1 and E4 in Picture 1a) are maintained at non-zero potentials. As a result, an electric double layer (EDL) is formed on both excitation electrodes. No direct contact is established between the grounding point and the investigated biological sample. The current flowing between electrodes E1 and E4 originates exclusively from the potential difference generated by the signal source.

In the equivalent circuit model [5], the double layer formed on electrodes E1 and E4 is represented by simplified Randles cells, which are constructed from Z_{CPE1}, R_{E1}, Z_{CPE4}, R_{E4} and Z_S elements, as it can be seen in Picture 1b. Here, Z_S represents the total impedance of the solution, while Z_{CPE1} and Z_{CPE4} denote the capacitive constant phase element (CPE) components at the excitation electrodes. Since the effect of the electric double layer is primarily concentrated in the immediate surrounding area of the electrodes (decreasing exponentially with distance) measuring electrodes (E2 and E3) are physically separated [5], [7], as it can be seen in Picture 1a. This tetrapolar arrangement allows cell and electrolyte impedance to be examined selectively while contact resistances are suppressed.



(a) **(b)**

Picture 1. *Schematic of the developed in vitro BIS technique (a) custom-made graphene plate, (b) the associated equivalent circuit model [5]*

The operating principle is based on frequency-dependent current distribution. At low excitation frequencies, cell membranes act as insulators, and current flows primarily through the extracellular space. In this range, the measured impedance is composed of the extracellular resistance (R_0) and the medium impedance. As frequency is increased, the capacitive behavior of the membrane becomes more pronounced, allowing the electric field to penetrate the intracellular space. This enables the structural and dielectric properties of the cell layer to be investigated in real-time, in a non-invasive way.

The bioimpedance spectrum (Z_{cell}) was calculated using the potential values u_2, u_3 and u_4 according to the formula:

$$Z_{cell} = R_{ref} \frac{u_2 - u_3}{u_4} \quad (1)$$

The measuring system is based on a high-precision digital lock-in amplifier with the following specifications: 10 V peak-to-peak maximum voltage, 1 mA maximum current, a range of 1 mHz–100 kHz, 64-bit IEEE 754 precision, <1 ppm amplitude accuracy, and 6,5 hours of battery life [5].

2. Experiments and Results

For the *in vitro* studies, measuring plates developed by the research group were utilized [5]. To optimize cell adhesion and ensure a stable measurement environment, Fibronectin and Poly-D-lysine coatings were applied to the surface of the measuring plates [8]. The application of these coatings establishes the foundation for the stability and reproducibility of subsequent bioimpedance-based cellular assays [9], [10]. A total of 20 measuring plates were prepared for the experiments, including 10 plates coated with Fibronectin and 10 coated with Poly-D-lysine. Validation of these plates was initially conducted using 2,5 ml volumes of physiological saline series (0%, 25%, 50%, 75%, and 100% concentrations) pipetted in ascending order. For the primary BIS measurements, 2,5 ml of solution from each of the six investigated concentrations were placed into the Petri dishes. These measurements were performed within a frequency range of 0,1–10 kHz and were repeated five times in succession for each sample.

For the experiments, two types of polystyrene bead suspensions were used, with particle diameters of 0,7 µm and 15,5 µm. These specific sizes were selected as extreme values based on their cellular interaction: while 0,7 µm particles are capable of entering the cells, the 15,5 µm beads exceed the size threshold required for uptake. Consequently, these two diameters represent the endpoints of the examined range.

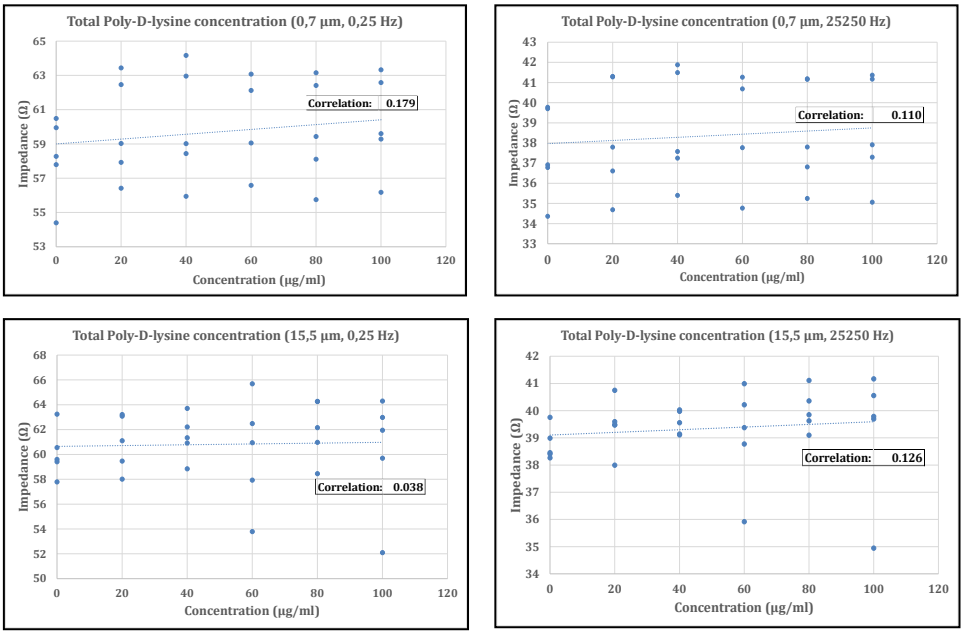
A concentration series of 0, 20, 40, 60, 80, and 100 µg/ml was established by mixing polystyrene (PS) bead suspensions (PS density: 1,05 g/cm^3) with physiological saline. From these mixtures, 2,5 ml aliquots were pipetted into the coated plates in a randomized sequence to minimize systematic error. BIS measurements were conducted using silver wire electrodes, with five replicates recorded for each concentration on every plate. Process illustrations are presented in Picture 2.



Picture 2. *BIS experimental configuration: (a) integrated measurement apparatus, (b) nanoplastic concentration series*

Impedance spectra were evaluated across the full frequency range, highlighting six key frequencies and comparing results with uncoated controls. In the present analysis, only the results of Poly-D-lysine coated plates were chosen to be presented, illustrating both particle sizes at representative low and high frequencies in Picture 3. Data were categorized by coating type, particle size, and concentration. To ensure measurement reliability, Pearson correlation analysis was performed between frequency points and repetitions, shown in Picture 3. Data processing was followed by the methodology of Vizvári et al. (2024) [5], focusing on relevant amplitude and phase values in order to identify frequency-dependent characteristics and measurement consistency.

In the diagrams grouped by experimental parameters, BIS measurements are presented as individual data points, each supplemented with a linearly fitted trendline and the calculated Pearson correlation coefficient. This analysis was employed to investigate the relationship between impedance and microplastic concentration, where the consistently low correlation values (ranging from 0.038 to 0.179, as it is labeled for each condition in Picture 3), no correlation was observed for either bead size. On Poly-D-lysine coated plates, measurement points from the five individual plates show a scattered distribution at all concentration levels.



Picture 3. *Pearson correlation of 0,7 and 15,5 µm beads on Poly-D-lysine coating (0,25 Hz and 25,25 kHz)*

This vertical dispersion is found to be frequency-dependent, with significantly higher variance observed at lower frequencies (0,25 Hz) compared to higher frequencies (25,25 kHz). For the 15,5 µm particles, the data points show a tighter clustering at multiple frequencies, indicating higher inter-plate consistency compared to the 0,7 µm beads.

3. Conclusions

The study aimed to assess whether the BIS method and surface coatings (Poly-D-lysine, Fibronectin) could enhance the detectability of micro- and nanoplastic particles. Results indicated that no clear, monotonic, or concentration-dependent impedance change was observed for any coating or particle size. While frequency dependence proved to be stable and reproducible, impedance patterns were primarily determined by individual variations between the measuring plates. Larger beads (15.5 µm) showed more consistent behavior, whereas smaller particles (0.7 µm) were more sensitive to coatings and manufacturing variations. Overall, the concentration-impedance relationship was not found suitable for detection, and coatings did not enhance sensitivity, so the

method is not considered fully reliable in this form. Given that previous experiments followed the same methodology [6], it is concluded that neither suspended nor beads attached to the coating were detected, suggesting that observed differences originated solely from intracellular beads. To enhance sensitivity, alternative coatings for stronger particle fixation should be tested. Plate fine-tuning and data processing to isolate particle effects from inter-plate variance are also recommended.

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