

Alteration of zeta potential and cell viability in rat-derived L6 skeletal muscle cells and H9c2 cardiomyocytes: A study with submicron polystyrene particles

Zmiany potencjału zeta oraz żywotności komórek mięśni szkieletowych L6 oraz kardiomiocytów H9c2: badania z submikronowymi cząstkami polistyrenowymi

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Abstract

Background. Micro- and nanoplastics pollution can cause substantial damage to ecosystems. Since scientists have focused mainly on their impact on aquatic environments, less attention has been paid to the accumulation of polymer particles in terrestrial organisms.

Objectives. We checked if submicron (<5 μm) polystyrene (PS) particles, which can accumulate in living organisms, lead to changes in the physicochemical properties of mammalian cell membranes.

Materials and methods. The influence of submicron PS particles on the properties of rat-derived L6 myocytes and H9c2 cardiomyocytes was analyzed. Non-functionalized and amine-functionalized PS particles of 100 nm and 200 nm in diameter were used. The MTT assay was performed to evaluate the viability of the polymers-treated cells. The effect of short (6 h) and prolonged (48 h) incubation with different concentrations of PS particles on the cell's zeta (ζ) potential was examined with the electrophoretic light scattering technique (ELS). Polystyrene particles' physicochemical characteristics (size and stability) were performed using dynamic light scattering (DLS) and electrophoretic light scattering methods.

Results. The results show that submicron PS particles affect cell viability and cause changes in the physicochemical parameters of rat cell membranes. Differences were observed depending on the origin of the cells. We observed dose- and time-dependent alterations in the studied parameters after submicron PS particle incubation in L6 myotubes and H9c2 cardiomyocytes.

Conclusions. The size and modification of PS particle surfaces determine the extent to which they affect the analyzed properties of rat cardiomyocytes and myocytes membranes.

Keywords: physicochemical properties, zeta potential, cardiomyocyte, myotubes, submicron polystyrene particles

Streszczenie

Wprowadzenie. Zanieczyszczenia mikro- i nanoplastikami mogą powodować znaczne szkody w ekosystemach. Ze względu na to, że naukowcy skupili się głównie na wpływie polimerów na środowisko wodne, mniej uwagi w literaturze poświęcono problemowi akumulacji ich cząstek w organizmach lądowych.

Cel pracy. Zbadano, czy submikronowe (< 5 mm) cząstki polistyrenowe, które mogą kumulować się w organizmach żywych, powodują zmiany właściwości fizykochemicznych błon komórkowych ssaków.

Materiał i metody. Przeanalizowano wpływ submikronowych cząstek polistyrenowych na właściwości szczurzych komórek mięśni szkieletowych L6 oraz kardiomiocytów H9c2. Zastosowano cząstki polistyrenu niesfunkcjonalizowanego i sfunkcjonalizowanego grupą aminową –NH₂ o średnicy 100 i 200 nm. W celu oceny żywotności komórek traktowanych polimerami przeprowadzono testy MTT. Wpływ krótko- (6 godzin) i długotrwałej (48 godzin) inkubacji z różnymi stężeniami cząstek polistyrenu na wartość potencjału zeta (ζ) zbadano techniką elektroforetycznego rozpraszania światła (ELS, ang. Electrophoretic Light Scattering). Charakterystykę fizykochemiczną (wielkość i stabilność) analizowanych cząstek polistyrenu określono metodami dynamicznego rozpraszania światła (DLS, ang. Dynamic Light Scattering) oraz ELS.

Wyniki. Uzyskane dane wykazały, że submikronowe cząstki polistyrenowe wpływają na żywotność szczurzych komórek mięśni szkieletowych oraz komórek mięśnia sercowego, a także powodują zmiany parametrów fizykochemicznych ich błon. Odnotowano różnice w zależności od rodzaju tkanki z której pochodzą komórki (mięśnie szkieletowe/mięsień sercowy). Uzyskane wyniki zależały od rodzaju i dawki polistyrenu, a także od czasu inkubacji.

Wnioski. Wielkość i modyfikacja powierzchni cząstek polistyrenu jest istotna dla stopnia w jakim wpływają one na właściwości fizykochemiczne błon szczurzych miotub oraz kardiomiocytów.

Słowa kluczowe: właściwości fizykochemiczne, submikronowe cząstki polistyrenowe, potencjał zeta, kardiomiocyty, komórki mięśni szkieletowych

Background

The main components of plastics are natural or synthetic polymers.¹ In the past, it was believed that plastics were the material of the future, making everyday life easier. However, due to their inappropriate use and management, they are now considered a severe environmental issue worldwide.^{2,3} The raw material used in the production of plastics is polystyrene (PS), a crucial compound, e.g., during the COVID-19 pandemic since most of the packaging in which meals were delivered to households was made from PS.⁴ This polymer is not biodegradable, and its disposal is a complicated and expensive process, the price of which exceeds the cost of producing the raw material.⁵ In addition to the improper utilization of plastic waste, which is essential for the future of our environment, the accumulation of plastics in living organisms is a fundamental issue. Submicron particles (smaller than 5 mm) may accumulate in the living organism. The particles are formed by the degradation of plastics into smaller and smaller materials,⁶ which may come from intentional production (primary plastics) or may be the products of the fragmentation of larger pieces (secondary plastics).⁷ Most polymer particles are classified as secondary plastics, and their fragmentation can occur as a result of exposure to O₂, temperature, ultraviolet (UV) radiation, or mechanical abrasion.⁸ Polymer particles are categorized as anthropogenic pollutants that are present all over the Earth, found in waters,⁹ soils¹⁰ and air.¹¹ Plastic pollution can cause substantial damage to ecosystems. Since scientists have focused mainly

on their impact on aquatic environments,^{12,13} less attention has been paid to the accumulation of polymer particles in terrestrial organisms. The literature reports the adverse effects of microplastics on mammals, such as impaired reproduction or changes in metabolism.^{14–16} Polymer particles can bioaccumulate in the organism and exhibit various levels of toxicity that can be attributed to their physicochemical characteristics (size, shape, surface chemistry), which may allow them to enter cells and interact with their components. This process can lead to nanoparticle-induced biophysical and/or biochemical changes.¹⁷

Objectives

A crucial issue worth focusing on is the effect of polymer particles on changes occurring within the biological membranes of organisms. *Rattus norvegicus* is used in biomedical research and remains the model of choice for chemical toxicity studies.¹⁸ Rat cells (L6 skeletal muscle cells and H9c2 cardiomyocytes) were chosen as an in vitro model system to investigate how anthropogenic pollutants, such as plastics, affect the properties of mammalian cell membranes. Since PS is one of the polymers that mainly pollute our environment and does not form in reactive forms in which cells occur (thus not damaging the membrane due to oxidative stress), its particles were chosen for the study.¹⁹ Non-functionalized (pristine) PS and amine-functionalized PS-NH₂ particles of 100 nm and 200 nm in diameter were used. Rat L6 myotubes and H9c2

cardiomyocytes were exposed to 6 h and 48 h of incubation with PS particles at concentrations ranging from 2 µg/mL to 1000 µg/mL.

In order to investigate the cytotoxic activity of PS particles toward L6 and H9c2 cells, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) biochemical assays were performed. Electrochemical techniques were used to examine the effect of PS particles on the electrical parameters characterizing the cell membranes after conducting biological tests. A significant parameter describing biological cells is the zeta potential (electrokinetic potential (ζ)) – an essential and credible index of the membrane's surface charge. Its value is characteristic of a membrane composition and knowledge of ζ makes it possible to determine the system's stability.²⁰ This parameter depends on viscosity, temperature and pH, so even slight modifications of the analyzed system may affect its value.²¹

Materials and methods

Materials

Cell culture

The study was conducted on the L6 skeletal muscle cells and H9c2 cardiomyocytes obtained from the American Type Culture Collection (ATCC; Manassas, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; PAN-Biotech, Aidenbach, Germany) with the addition of 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, USA) and 1% solution of antibiotics (PAN-Biotech) in a humidified atmosphere containing 5% CO₂ at 37°C. Confluent cells were then cultured in differentiation medium containing DMEM and 2% horse serum (HS; Thermo Fisher Scientific) for L6 cells and DMEM enriched with 1% of FBS for H9c2 myoblasts. After 2 weeks, myoblasts were fully differentiated into elongated, multinucleated myotubes. Thereafter, myotubes were exposed to PS nanoparticles (PS/PS-NH₂) of 100 nm or 200 nm in diameter at concentrations ranging from 2 µg/mL to 1000 µg/mL and incubated for 6 h and 48 h. Myotubes cultured without PS/PS-NH₂ were used as a control.

Polymers

Pristine 100-nm and 200-nm PS (Sigma-Aldrich, Saint Louis, USA), amine-functionalized 100-nm (Polysciences, Hirschberg an der Bergstrasse, Germany) and 200-nm (Bang Laboratories, Fishers, USA) PS-NH₂ were used to treat L6 and H9c2 cells. The amount of dose and the time of treatment with the PS particles were chosen in the pre-study. The PS molecules were diluted to the required concentration with 155 mmol/L NaCl, in which the cells were also suspended.

Methods

Cell viability assay

Cytotoxicity of PS nanoparticles was assessed with MTT viability assay (Sigma-Aldrich). Briefly, L6 and H9c2 cells were cultured on 96-well plates at a density of 4×10³ cells/well. Fully differentiated myotubes were incubated with different concentrations of PS/PS-NH₂ ranging from 2 µg/mL to 1000 µg/mL for 6 h and 48 h. Cells were then rinsed 2 times with PBS and incubated with 200 µL of MTT solution (5 mg/mL in PBS) for 4 h. After removing the medium, the purple precipitate was dissolved in 200 µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich). The absorbance of the resulting formazan solution was measured at 570 nm using a microplate reader (Synergy H1 Hybrid Reader; BioTek Instruments, Winooski, USA). The viability of pristine PS/PS-NH₂-treated cells was calculated as the percentage of untreated cells.

Dynamic light scattering

The dynamic light scattering (DLS) technique was used to estimate the diameter of DMEM-suspended polymer particles (pH = 7.4, T = 25°C). Measurements were made with the Zetasizer Nano ZS analyzer (Malvern Instruments Ltd, Malvern, UK). Applying the Stokes–Einstein equation, the particle velocity caused by Brownian motion is transformed into a particle size distribution. Using a non-invasive backscattering technique, in which the detector is angled at 173°, particle size detection can be done. The derived data were error-laden, represented as standard deviation.

Electrophoretic light scattering

Zetasizer Nano ZS analyzer uses the electrophoretic light scattering (ELS) method to determine the ζ potential of the PS particles and cells. In the first step, the parameter for both 100-nm and 200-nm PS/PS-NH₂ was measured (DMEM, pH = 7.4, T = 25°C). In the next step, L6 and H9c2 cells were treated with both pristine and amine-functionalized PS particles (2 µg/mL, 10 µg/mL and 100 µg/mL) for 6 h and 48 h in 155 mmol/L NaCl, and ζ potential was measured as a function of pH (pH range 2.5–10). The microelectrophoretic measurements were also performed for control samples – L6 and H9c2 cells suspended in 155 mmol/L NaCl untreated with PS. At least 3 repeated measurements on each sample were taken to check for result repeatability.

Statistical analyses

GraphPad Prism 9 software (GraphPad Software, San Diego, USA) was applied to statistically analyze the data derived from the MTT test. The data that fulfilled normality

and homogeneity assumptions were analyzed using a one-way analysis of variance (ANOVA). If the abovementioned assumptions were not fulfilled, we applied the Kruskal–Wallis test. As a post hoc test, Dunnett's multiple comparisons test (parametric test) or Dunn's multiple comparisons test (non-parametric test) were used. Statistical significance was defined as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Data obtained from the DLS and ELS studies were given as means \pm standard deviation ($M \pm SD$). The data were evaluated with standard statistical analyses, namely one-way ANOVA with Scheffe's F test for multiple comparisons to detect significance between the various groups. Values of $p < 0.05$ were assumed to be relevant.

Results and discussion

The size and stability of submicron polystyrene particles

Measurements of both DLS and ELS have been used to evaluate the PS particle changes in size, charge (ζ potential) and particle size distribution (polydispersity index (PDI)) in DMEM (a medium in which L6 and H9c2 cells were cultured). During the experiments, 100-nm and 200-nm non-modified (PS-100 and PS-200) and amino-functionalized PS particles (PS-NH₂-100 and PS-NH₂-200) were analyzed.

Particle size distribution curves (by number and intensity) are presented in Fig. 1. As can be seen from Table 1, the sizes by number of PS-NH₂ (both 100-nm and 200-nm) are consistent with the commercially stated values and comparable to results obtained in 155 mmol/L NaCl.²² Analyzing the data registered for non-modified PS submicron particles, we can see that PS with 200 nm in diameter dispersed in DMEM deviate from the commercially stated values and are equal to 297.70 ± 118.90 nm (size by number) and 387.40 ± 147.00 nm (size by intensity). The obtained differences may be caused by the stronger dispersion of PS particles in DMEM than in sodium chloride.²³ Moreover, PS-100 suspended in DMEM exhibits a bimodal size distribution profile (Fig. 1), with PDI = 0.328, indicating that pristine PS particles are polydisperse. This proves the tendency of PS-100 to form aggregates in DMEM, a common phenomenon characterizing PS nanoparticles.

The ζ -potential characterizes the electrokinetic potential of nanoparticles in solutions, which is a factor that alters the stability of particles and thus their possible absorption or toxicity.²⁴ In general, if ζ is higher than 30 mV, negative or positive, it suggests high physical stability of the system because of the electrostatic repulsion of the particles.²⁵ A ζ value between -30 mV and $+30$ mV usually suggests flocculation and/or aggregation of the particles.²⁶ From the data summarized in Table 1, it may be deduced that amine-modified PS particles show lower stability than native particles. Non-functionalized PS particles have

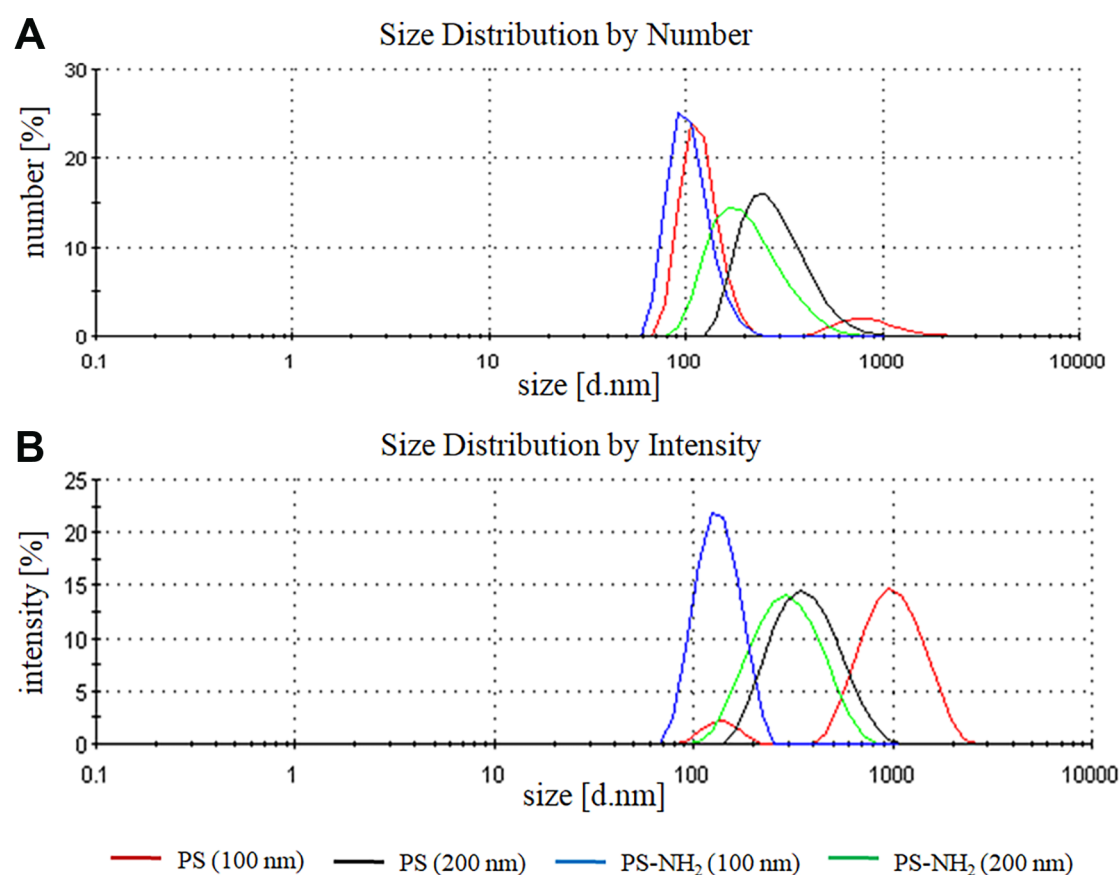


Fig. 1. Polystyrene (PS) size distribution by (A) number and (B) intensity in Dulbecco's modified Eagle's medium (DMEM; $C_{PS} = 100 \mu\text{g/mL}$, $\text{pH} = 7.4$)

Table 1. Polymers physicochemical characteristics (DMEM, $C_{PS} = 100 \mu\text{g/mL}$, pH = 7.4)

Polymer	Size by number [nm]	Size by intensity [nm]	PDI	ζ potential [mV]
PS (100-nm)	119.40 \pm 25.90	139.00 \pm 26.61	0.328	–22.08 \pm 0.68
PS (200-nm)	297.70 \pm 118.90	387.40 \pm 147.00	0.183	–31.18 \pm 1.06
PS-NH ₂ (100-nm)	106.70 \pm 26.69	135.40 \pm 32.44	0.250	–9.62 \pm 0.42
PS-NH ₂ (200-nm)	215.80 \pm 94.18	310.30 \pm 119.70	0.126	–15.73 \pm 0.72

DMEM – Dulbecco's modified Eagle's medium; PDI – polydispersity index; PS – polystyrene.

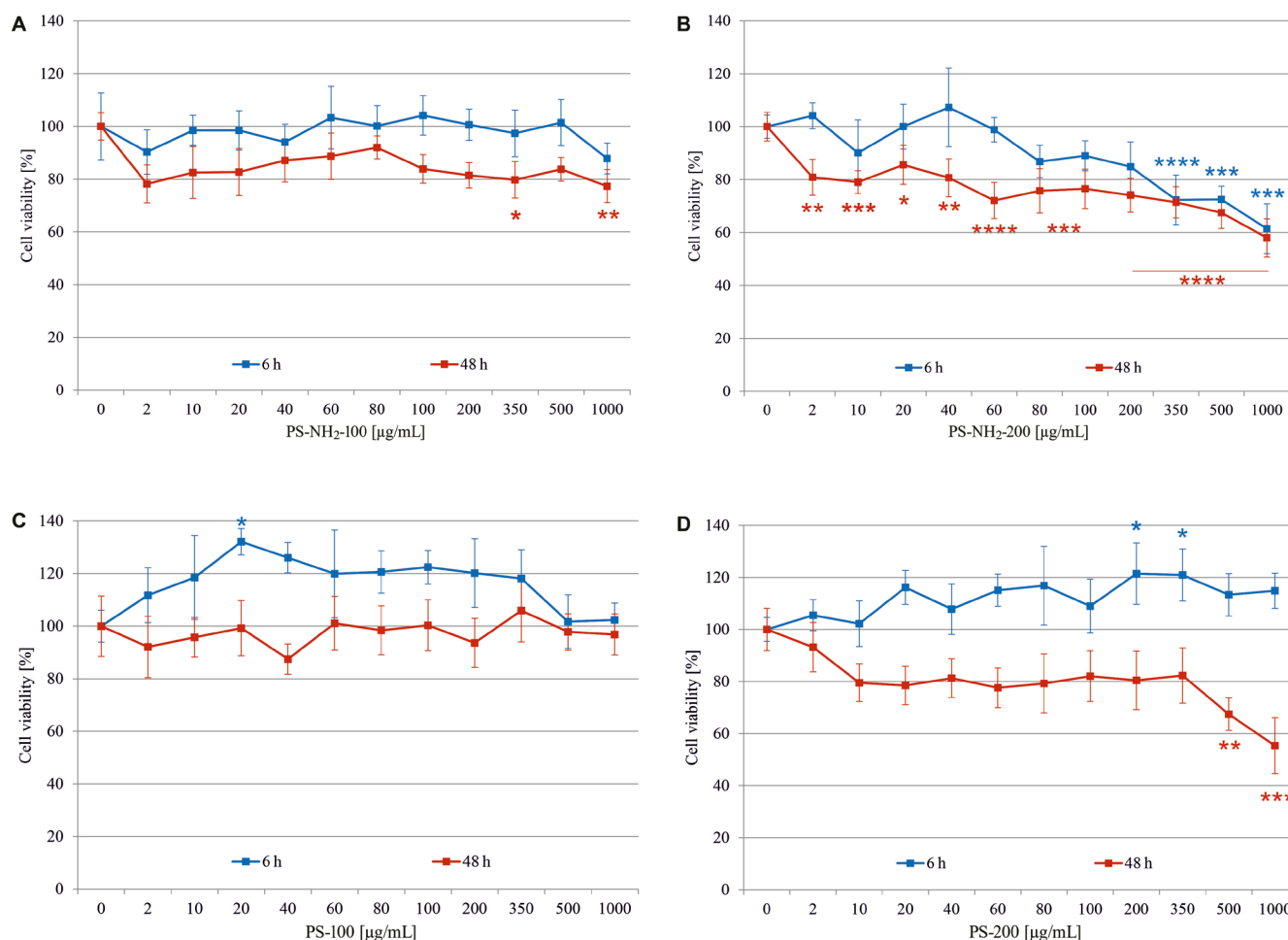


Fig. 2. The effect of different types of polystyrene (PS) particles on the viability of L6 cells. Cells were incubated for 6 h and 48 h with the indicated particle concentrations. Data are represented as the percentage of counted cells relative to the untreated control. Statistical significance was defined as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

more negative ζ -potential of -31.18 ± 1.06 mV (PS-200) and -22.08 ± 0.68 mV (PS-100) compared to amine-modified ones (-15.73 ± 0.72 mV (PS-NH₂-200) and -9.62 ± 0.42 mV (PS-NH₂-100)). This can be explained by the fact that the presence of amino groups causes their interactions with the medium. This confirms that the presence of -NH₂ groups in the system increases its tendency to combine into larger assemblies.²⁷ Moreover, both pristine and amino-functionalized PS particles show significantly lower stability when dispersed in DMEM than in 155 mmol/L NaCl, directly increasing the chances of the particles to form aggregates, which was shown in the case of 100-nm pristine PS.²²

The influence of submicron polymer particles on cell viability

The cytotoxic effect of submicron PS particles on rat L6 myotubes and H9c2 cardiomyocytes was assessed using MTT assay. Cells were incubated with native and amine-functionalized 2 sized PS molecules (100-nm and 200-nm) at concentrations ranging from 2 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$ for 6 h and 48 h. The results for the L6 cell line are presented in Fig. 2, and data obtained for the H9c2 line are collected in the Supplementary data.

As shown in Fig. 2A, incubation of the cells with PS-NH₂-100 did not induce cytotoxicity at least up

to concentrations of 350 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ after 48 h of incubation. PS-NH₂-200 (Fig. 2B) exerted the most severe cytotoxic effect on L6 cells, especially after 48 h of treatment. The lowest dose of PS-NH₂-200 induced a mild cytotoxic impact reaching 80% cell viability. Incubation with increasing concentrations of PS-NH₂-200 (i.e., 350 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$) exhibited more pronounced cytotoxic effects, approaching 28.6%, 32.5% and 42% non-viable cells after 48 h, respectively. Similarly, incubation for 6 h with the highest concentrations of PS-NH₂-200 led to a decrease in L6 cell survival, reaching from 27.7% to 38.5% of non-viable cells (Fig. 2B). This is presumably because particles containing amino groups in their structure affect cell signaling pathways, thereby influencing proliferation and accelerating the process of apoptosis.²⁸ Many biochemical processes describing signals from outside the cell leading to these changes have not yet been thoroughly investigated and reported in the literature.²⁹ Polystyrene particles containing -NH₂ groups with 100 nm in diameter affect cell proliferation to a lesser extent.

Moreover, PS-100 had a relatively low ability to limit cell viability and even had a growth-promoting effect after 6 h of incubation (Fig. 2C). Pristine 200-nm PS also had a growth-stimulatory impact at 6 h; however, a cytotoxic effect was observed for 500 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ at 48 h (Fig. 2D). This is confirmed by a few *in vitro* studies where the cytotoxic effects of PS particles were compared in mammalian cell culture systems representing organs belonging to different potential entry pathways. It has been reported that amine-modified molecules show higher toxicity in various cell lines, inducing reactive oxygen species (ROS), cell death, the damage of mitochondria, and genotoxicity, while no cytotoxicity was observed with unmodified molecules.^{30–32}

On the other hand, H9c2 cardiomyocytes exposed to both unmodified or amine-modified PS nanoparticles showed significantly greater viability than L6 cells (Supplementary Fig. 1). The cytotoxic effect was observed solely for the highest concentration (1000 $\mu\text{g/mL}$) of tested PS nanoparticles, except PS-100, after 48 h of incubation.

Our results revealed that mainly 200-nm NH₂-modified nanoparticles induced severe toxicity after 48 h of incubation, albeit only to L6 cells. Moreover, the dose-dependent loss of viability was most pronounced in this case.

The influence of submicron polymer particles on the ζ potential of rat cells

The ELS experiment was intended to indicate possible changes in the ζ potential of rat cells treated with PS particles compared to the control samples. The measurements were performed after 6-h and 48-h incubation of L6 cells with PS particles. The effect of both pristine and amino-functionalized 100-nm and 200-nm PS particles on ζ of L6 cells in a function of pH of electrolyte (155 mmol/L NaCl) was analyzed. The data obtained for L6 cell line are presented in Fig. 3, 4, and for H9c2 cell line – in Supplementary

Fig. 2 and Supplementary Fig. 3. Furthermore, in the Supplementary data, a statistical analysis of the results obtained for L6 cell line treated with PS-NH₂ is presented (Supplementary Table 1 and Supplementary Table 2).

The profiles of all curves collected in Fig. 3 are similar: positive ζ values are observed in an acidic environment, and negative ζ values are observed in an alkaline environment. The most visible changes in the electrokinetic potential values were observed after exposure of the cells to PS-NH₂-100 (100 $\mu\text{g/mL}$) compared to untreated ones. After 6-h incubation (Fig. 3A), a decrease in ζ potential values was observed in acidic pH (pH = 2.5–4.5), where the ζ potential reached the highest value (compared to control) of 13.10 ± 0.74 mV. Slight changes in ζ in pH range of 6.5–10.0 were noted. In the same pH range, a more pronounced decrease in negative ζ value was observed after 48 h of exposure of skeletal muscle cells to PS-NH₂-100 particles (Fig. 3B). Furthermore, after both 6 h and 48 h of the cell's exposure to 100 $\mu\text{g/mL}$ PS-NH₂-200 particles, in an acidic pH (2.5–4.0) statistically significant changes in ζ potential values were noted (Supplementary Table 2). In general, due to the treatment of analyzed cells with both PS-NH₂-100 and PS-NH₂-200, more significant changes in ζ were observed after 48 h of incubation of the L6 cells with polymer particles, which may suggest that amine-modified PS particles do not penetrate the cell membrane after 48 h but adsorb on their surface. The surface charge is the parameter that can explain the changes occurring on the membrane surface. The surface of animal cell membranes is negatively charged as a result of the occurrence of, among others, sialic acid residues in membrane-building carbohydrate chains.³³ Cationic groups, such as amino groups, have been proven to interact with the negatively charged membrane.³⁴ The interactions lead to electrostatic attraction in the system and cause lysis of the cell membrane, allowing positively charged particles to penetrate the membranes more easily. Moreover, inert particles were found to penetrate the membrane to a lesser extent.^{34,35}

The effect of PS-100 and PS-200 on the ζ potential of L6 cells in a function of pH was also analyzed; experimental curves are presented in Fig. 4. The profiles of curves presented in Fig. 4 are similar to those obtained for PS-NH₂ particles (Fig. 3). Based on the data obtained, it was concluded that no statistically relevant alterations were observed in the ζ potential of the cells over the entire pH range for the PS-200 treatment. However, incubations of the cells with PS-100 lead to slight changes in ζ potential values both in acidic and basic pH after 6 h and in acidic media after 48 h. It is worth noting, however, that changes in a strongly acidic environment can be due to the membrane structure destruction caused by the concentration of H₃O⁺ ions.

The electrokinetic potential depends on the level of ionization of functional groups of particles like amino groups (-NH₂).³⁶ On the other hand, the degree of ionization of amino groups depends on the pH and ionic strength of the electrolyte. The amino groups are positively charged

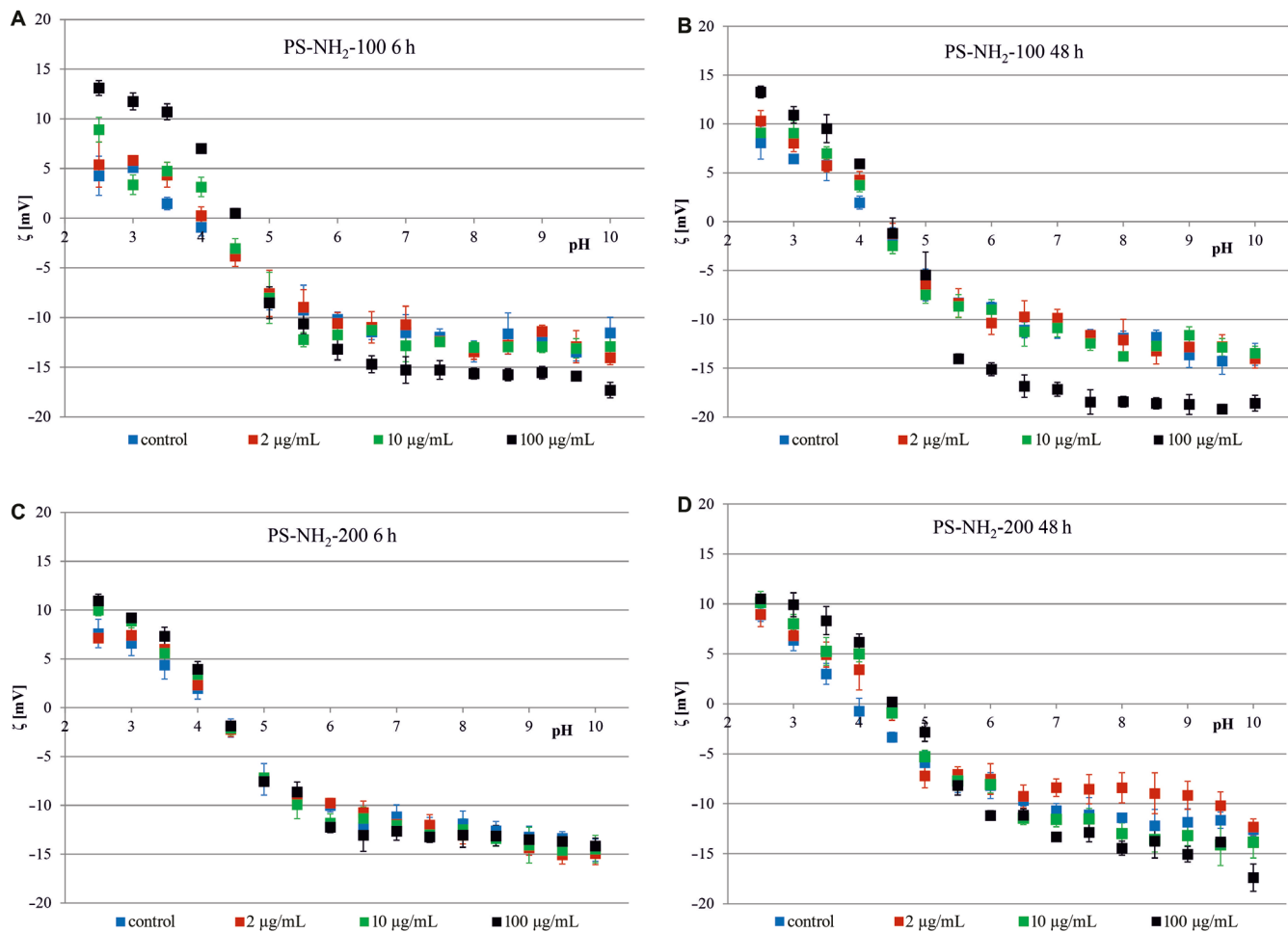


Fig. 3. The zeta (ζ) potential of L6 cells as a function of the pH of the electrolyte solution. Control cells and cells treated with 2 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ of polystyrene (PS) and after various times of incubation of the cells in the presence of polymer particles: A. PS-NH₂-100, 6 h; B. PS-NH₂-100, 48 h; C. PS-NH₂-200, 6 h; and (D) PS-NH₂-200, 48 h. Statistical analysis is presented in Supplementary Table 1

(-NH₃⁺) at pH below the isoelectric point. Above the isoelectric point, the groups become largely neutral (-NH₂), and ζ tends to decrease as the OH⁻ ions begin to react with the neutral amino groups, leading to a negative charge in the solution.^{37,38} Such variations cause variations in ζ values due to electrostatic shielding and the binding ion effect.³⁹

Interactions of polymer particles with cell membranes are an extremely complex phenomenon that depends on the size and shape of particles, the surface composition of the components that make up the system, or the surface charge that forms on membrane surfaces.^{37,38} Various behaviors of PS particles in relation to biological structures have been described in the literature. Polymers can interact with each other by several mechanisms, such as hydrogen bonds, hydrophobic interactions or van der Waals forces.^{40,41}

Conclusions

Nowadays, there is a strong necessity to understand how polymer particles affect the properties of living cells. The physicochemical properties of nanomaterials, including

polymers, contribute to their behavior within the biological milieu. Surface charge and particle size are the most frequently cited factors responsible for various biological effects of nanoparticles, including toxicity or cellular uptake. We have shown that PS particles influence rat cardiomyocytes and myotubes' viability and ζ potential. Moreover, our studies indicate that the polymer particles' size and surface modifications (amine-functionalized) define the extent to which they influence the physicochemical behavior of cell membranes. The results also depend on the polymer particle concentration and the time the cells are incubated with PS. It should be emphasized that, in recent years, studies on the influence of submicron polymer particles on laboratory animals are becoming more frequent. Polymer molecules can overcome the organism's barriers and therefore penetrate and accumulate in organs and tissues. The physicochemical properties, surface modifications of PS submicron particles and their large surface area promote their interaction with cell membranes, leading to internalization of the polymer by cells. We hope that our research may provide evidence for the behavior of the PS particles in rat cells, and contribute to a better understanding of their potential

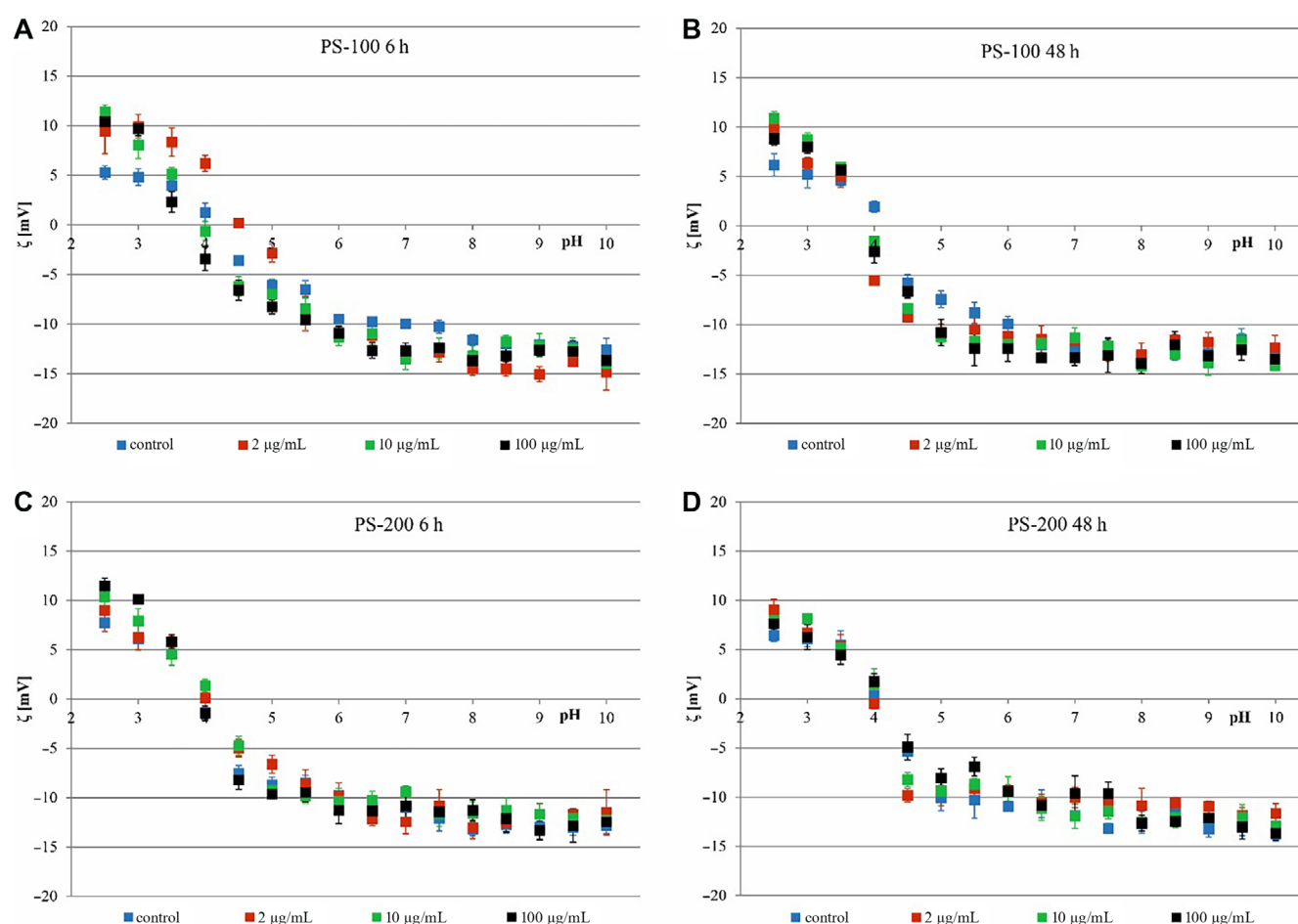


Fig. 4. The zeta (ζ) potential of L6 cells as a function of the pH of the electrolyte solution. Control cells and cells treated with 2 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ of polystyrene (PS) and after various times of incubation of the cells in the presence of polymer particles: A. PS-100, 6 h; B. PS-100, 48 h; C. PS-200, 6 h; and (D) PS-200, 48 h. Statistical analysis is presented in Supplementary Table 2

harm to organisms to avoid the risk of some diseases. Nevertheless, more in-depth studies are needed to further elucidate the mechanisms of interaction between PS particles and the cell membrane of animals.

Supplementary data

The Supplementary materials are available at <https://doi.org/10.5281/zenodo.10401147>. The package includes the following files:

Supplementary Fig. 1. The effect of different PS particles on the viability of H9c2 cells.

Supplementary Fig. 2. The ζ potential of H9c2 cells treated with amine-functionalized PS particles in a function of pH.

Supplementary Fig. 3. The ζ potential of H9c2 cells treated with pristine PS particles in a function of pH.

Supplementary Table 1. The ζ potential of the L6 cells after exposure to PS-NH2-100. Measurements were performed after 6 h and 48 h.

Supplementary Table 2. The ζ potential of the L6 cells after exposure to PS-NH2-200. Measurements were performed after 6 h and 48 h.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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