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The Effect of Modern Lifestyle on Cardiovascular Health

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Abstract. Polyethylene terephthalate (PET), a widely used microplastic, has been detected in the human cardiovascular system and raised safety concerns in the recent decade, yet its impact on vascular health remains poorly defined. Here, we investigated PET-induced endothelial dysfunction and the protective role of Sirtuin 1 (SIRT1). Human umbilical vein endothelial cells (HUVECs) were treated with PET (0–1000 μg/ml, 24–72 h) with or without SIRT1 modulation. PET exposure reduced viability, increased apoptosis, suppressed endothelial nitric oxide synthase (eNOS), and elevated reactive oxygen species (ROS) in a dose-dependent manner. SIRT1 overexpression attenuated these impairments, whereas knockdown exacerbated them. Transcriptomic profiling revealed PET-induced activation of inflammatory pathways, disruption of lipid metabolism, and impaired cell growth and function, all of which were worsened by SIRT1 inhibition. These findings identify PET as a potent disruptor of endothelial survival and highlight SIRT1 as a critical protective factor. Targeting SIRT1 may provide therapeutic strategies against microplastic-associated cardiovascular disease.

Keywords: Microplastics, PET, Endothelial cells, SIRT1, Cytotoxicity

1. Introduction

Microplastic pollution has emerged as a critical environmental issue, with polyethylene terephthalate (PET) comprising a significant proportion of microplastic waste. Due to its thermostability, high strength, and resistance to moistures and chemicals feature, PET is the most found type of microplastic on Earth, making up to 6% of global plastic production [1]. However, PET is increasingly found in ecological systems. PET has been found in our vital organs, with its average concentration in the human serum reaching 1.6 μg/ml [2]. Common health concerns of PET includes birth problems, cancer, hormone changes, liver dysfunction, etc.

Recent studies have highlighted the potential health implications of microplastic exposure, raising concerns about their effects on cellular function and systemic health. Therefore, of particular interest is the vascular endothelium, a monolayer of cells lining blood vessels that plays a pivotal role in maintaining vascular integrity, regulating blood flow, and controlling inflammatory responses. Endothelial dysfunction, often characterized by reduced nitric oxide (NO) bioavailability, increased oxidative stress, and heightened inflammation, is a precursor to numerous cardiovascular diseases, including atherosclerosis and hypertension [3]. The molecular mechanisms underlying PET-induced endothelial dysfunction remain poorly understood.

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On the other hand, lifestyle interventions such as exercise have been shown to improve endothelial function through SIRT1 activation. Sirtuin 1 (SIRT1), a NAD+-dependent deacetylase, is a well-recognized regulator of endothelial function. By modulating key transcription factors such as NF-κB and promoting NO production via eNOS activation, SIRT1 exerts protective effects against oxidative damage [4]. Dysregulation of SIRT1 activity has been implicated in the progression of endothelial dysfunction and cardiovascular disease, making it a potential mediator in the vascular effects of PET microplastic exposure [5]. Exercise enhances vascular health by promoting NO bioavailability, reducing oxidative stress, and improving endothelial barrier function. The role of SIRT1 as a mediator of exercise-induced vascular benefits is well-established, but whether it can counteract the detrimental effects of PET microplastics on endothelial cells remains unexplored.

This study aims to address critical gaps in understanding how PET microplastics, and SIRT1 intersect to influence endothelial cell function. Specifically, we investigate (1) the impact of PET microplastic exposure on endothelial cell survival and function, (2) whether exercise-mimicking interventions that enhance SIRT1 activity can mitigate the adverse effects of PET on endothelial cells, and (3) the molecular mechanism of SIRT1-mediated vascular protection. This research provides novel insights into the molecular mechanism affected by environmental microplastics and highlights potential therapeutic strategies to protect vascular health under microplastic over-exposure.

2. Material & methods

2.1. Cell culture

Human Umbilical Veins Endothelial Cells (HUVECs) were used with MEC culture medium (ScIenCellTM , 1001) for cell culture. Cells were cultured in 5% CO2 and 37˚ Celsius environment with controlled humidity. Cells were passaged and expanded when reaching 90% cell density. Up to passage 10 were used for evaluation.

2.2. PET treatment

Stock PET solution (20mg/ml, ~100nm in size, Zhong ke ke you) was serial diluted in sterile water containing 0.05% BSA. The following concentrations were prepared: 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml. HUVECs were treated with PET at 24h , 48h , and 72h , respectively.

2.3. SIRT1 overexpression

Gain of SIRT-1 was achieved by transiently transfecting with SIRT-1 plasmids using Lipofectamine (Invitrogen Life Technologies, L3000001), according to the manufacturer's instructions. Empty plasmid was served as additional control group. Briefly, HUVECs were plated in T25 flasks and transfection was initiated when reaching 70-80% confluency. 1.8μL of plasmid was mixed with 9μL of Lipofectamine prior adding to cell culture plate. SIRT1 overexpression was evaluated by qPCR and Western Blot 48 hours post transfection.

2.4. SIRT1 siRNA knockdown

SIRT1 knockdown was achieved by transiently transfecting SIRT1 siRNA with Lipofectamine (Invitrogen Life Technologies, L3000001), according to the manufacturer's instructions. siRNA-NC was used as control group. Briefly, HUVECs were plated in T25 flasks and transfection was initiated

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when reaching 70-80% confluency. 15μL of siRNA was mixed with 9μL of Lipofectamine prior adding to cell culture plate. After 48 hours, total RNA and protein were extracted for evaluating SIRT1 knockdown efficiency by qPCR and Western Blot.

2.5. RT-qPCR

Cells were lysed by washing with PBS and adding RNA extraction solution (1mL) to 6-wells plate and 0.5mL to 12-wells plate. Then, 100 uL of chloroform substrate (Shan Dong Shuang Shuang Hua Gong, GB/T682-2002) was added per each mL of RNA extraction solution. After centrifuged in 4 degrees for 15 min, 500 uL of isopropanol (Tian Jin Zhi Yuan, 1010) was added. Then, the solution was centrifuged for 10 min in 4 degrees and 75% ethanol was added to solution. 20-50 uL DEPC water were added for RNA extraction.

The reverse transcription was conducted using a commercial reverse transcription kit (Novoprotein E047-01B) according to manufacturer's instructions. Briefly, 1μg of total RNA was used with reaction mix. Subsequently, samples were incubated at 50℃ for 15min,then 75℃ for 5min.

The cDNA product was subjected to qPCR using a commercial kit (Novoprotein E096-01A) according to manufacturer's instructions. Briefly, 1μL of total cDNA was used with reaction mix, the primer sequences are listed in Table X. Subsequently, samples were incubated at 95℃ for 5min, 1 cycle, then 95℃ for 10 seconds, 60℃ for 10 seconds, and 72℃ for 10seconds, 40 cycles. Data was plotted and analyzed using the 2-ΔΔct method.

Table 1. Forward and reverse primer sequences

Primer Name	Primer Sequence
GAPDH-Forward	ATTCCACCCATGGCAAATTCC
GAPDH-Reverse	GACTCCACGACGTACTCAGC
SIRT1-Forward	GTAGGCGGCTTGATGGTAATC
SIRT1-Reverse	TACCTCAGCGCCATGGAAAA

2.6. Western blot analysis

HUVECs were lysed in 6-wells plate with 100μL RIPA lysing solution with 10μL protease inhibitor (100mM) and 10μL PMSF (100mM). The cells were washed once with PBS after removing culture medium, followed by adding precooled lysis solution. Then, the lysates were centrifuged for 10 minutes at 4 °C, 12000 rpm. Protein concentration was measured by BCA assay kit according to manufacturer's recommendation. Samples were subjected to 12.5% SDS-PAGE analysis and electrophoresed at 80V, 15min, and 120V, 60min. Membranes were transferred at 150mA for 90 min, followed by treating with primary antibody (concentration: 1:1000, GADPH concentration 1:10000) and secondary antibody (HRP labeled goat anti-rabbit IgG dilution ratio 1:10000).

2.7. Cell viability assay

Cell Viability was measured by CCK-8 (SuperKine™ Abbkine, BMU106-CN) according to manufacturer's recommendations. Briefly, after treatment, 10 μL of CCK-8 working solution was added into each well and incubated for 2 hours. Absorbance was measured at 450nm by TECAN

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SPARK plate reader. Cell viability was calculated by subtracting the absorbance of blank wells containing only the medium and normalizing the resulting values to the untreated control group.

2.8. Cell apoptosis assay

Cell apoptosis was evaluated by Caspase-Glo® 3/7 Assay (Promega, G8091) accordingly to manufacture's recommendations. Briefly, after treatment, Caspase-Glo® 3/7 solution was added to HUVECs' culture medium in 1:1 ratio. After 1 hour of incubation, luminescence was tested with microplate reader (TECAN SPARK). The cell apoptosis was calculated by subtracting the absorbance of blank wells containing only the medium.

2.9. eNOs measurement by ELISA

eNOs level was measured with eNOs ELISA Kit (Cloud-Clone Corp, SEA868Hu) according to manufacturer's instructions. Briefly, HUVECs were placed in 96-well plate (100 μL/well, 4,000 cells/well) and pre-cultured for 24 hours. After transfecting with si-SIRT1 3 for 6 hours, cells were treated with PET microplastics at 1000 μg/ml for 72 hours. HUVECs cell lysate was collected by trypsin and eNOs concentration was measured by ELISA kit. The results were read at 450nm by TECAN SPARK plate reader.

2.10. ROS measurement

ROS was measured with ROS Assay Kit (Elabscience E-BC-K138-F) according to manufacturer's instructions. HUVECs were placed into a 6-well plate and pre-cultured for 24 hours. After being transfected with si-SIRT1 3 or vector control for 6 hours, cells were treated with PET microplastics at 1000 μg/ml and incubated for 72 hours. Cells were then washed with serum free medium, and a 10 μM solution was used. This incubation happened in a dark environment at 37c. Last, ROS levels were read at 500nm and 525nm with flow cytometry.

2.11. RNA sequencing

RNA was isolated from three biological replicates of HUVECs in each group (NC, PET + NC, and PET+siSIRT1), followed by preparation for RNA sequencing. Quality control was performed to ensure the quality of sequencing. DESeq2 was used to analyze the sequencing data.

3. Results

3.1. PET decreased cell viability in HUVECs

The impact of PET on endothelial cell viability was evaluated in HUVECs treated with increasing concentrations of PET (0, 1, 10, 50, 100, 200, 500, and 1000 μg/mL) for up to 72 hours. As a result, PET reduced cell viability in a dose-dependent ($P<0.001$) and time course dependent manner. ($P < 0.001$).

Notably, at 100 μg/mL, cell viability decreased to 93.51% at 24 hours ($P < 0.01$, Figure 1A) and 84.52% at 72 hours ($P < 0.01$, Figure 1C). At the highest concentration treated (1000 μg/mL), cell viability was reduced to 78.50% at 24 hours ($P < 0.01$, Figure 1A), 75.42% at 48 hours ($P < 0.01$, Figure 1B), and 55.96% at 72 hours ($P < 0.01$, Figure 1C). These results indicate a significant time- and concentration-dependent cytotoxic effect of PET on HUVECs.

DOI: 10.54254/2753-8818/2025.AU30222



Figure 1. HUVECs viability (normalized to untreated control) post PET treatment

(A) HUVECs cell viability under 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml after 24 hours. (B) HUVECs cell viability under 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml after 48 hours. (C) HUVECs cell viability under 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml after 72 hours. Data represented as Mean± SD. n=3, NS: none significant. * P<0.05, **P<0.01 versus control, ****P<0.0001 by one-way Anova and unpaired T-test.

3.2. PET increased cell apoptosis in HUVECs

The effect of PET on endothelial cell caspase 3/7 activity and apoptosis was assessed in HUVECs treated with PET at concentrations of 0, 1, 10, 50, 100, 200, 500, and 1000 μg/mL. Compared to the untreated control group, PET treatment resulted in a significant increase in apoptosis up to 124% (P <0.0001, control vs 500 μg/ml, 48 hours; Figure 2B). At the highest concentration tested (1000 μg/mL), caspase-3/7 activity was elevated by 68%, 95%, and 124% after 24, 48, and 72 hours, respectively, relative to the corresponding control (P < 0.001, P < 0.0001, P<0.01; Figure 2A-C).

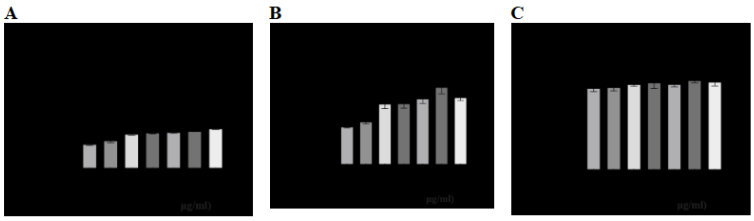

Figure 2. Caspase 3/7 activity in HUVECs post PET treatment

(A) Caspase 3/7 activity post 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml of PET after 24 hours. (B) Caspase 3/7 activity post 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml of PET after 48 hours. (C) Caspase 3/7 activity post 0, 1, 10, 50, 100, 200, 500, 1000 μg/ml of PET after 72 hours. Data represented as Mean± SD. n=3, NS: none significant. * P<0.05, **P<0.01, ****P<0.0001 versus control by one-way Anova and unpaired T-test.

3.3. Achievement of SIRT1 knockdown in endothelial cells using siRNA

To determine how SIRT1 affects PET-induced changes in endothelial cells, SIRT1 was knocked down. Three SIRT1 siRNA sequences were used to test knockdown efficiency. Compared to the control group, si-SIRT1 1 reduced SIRT1 gene expression by 9.24% (P=0.77); si-SIRT1 2 reduced SIRT1 gene expression by 30.26% (P<0.01); si-SIRT1 3 reduced SIRT1 gene expression by 56.47% (P<0.01) (Figure 3B). Taken together, the data indicated si-SIRT1 3 was most effective at decreasing SIRT1 level, therefore was selected for further protein evaluation in HUVECs, as a result, si-SIRT 3 reduced SIRT1 protein level by 66.72% (P<0.01, Figure 3C), further confirming the knockdown efficiency at protein level.

DOI: 10.54254/2753-8818/2025.AU30222

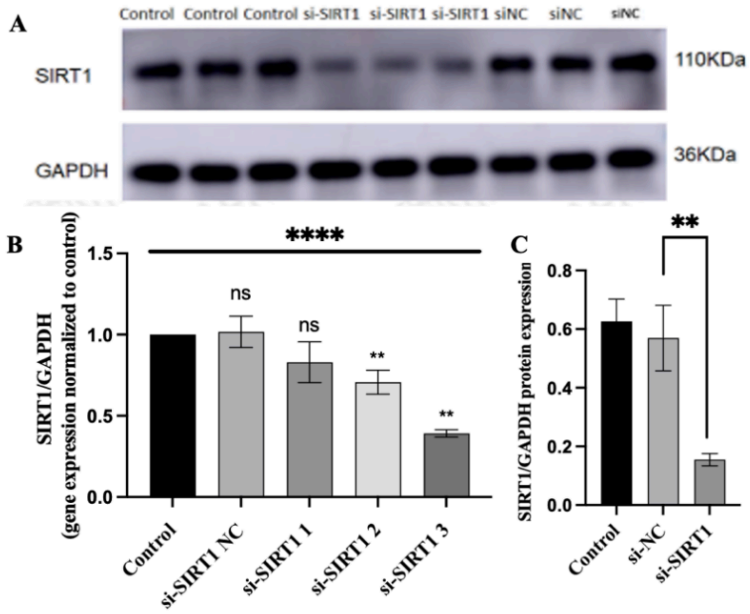

Figure 3. siRNA-mediated SIRT1 knockdown in endothelial cells

(A) Representative images of Western Blot showing SIRT1 protein expression after 48 hours post transfection. (B) SIRT1 gene expression was significantly down regulated with si-SIRT1 2 and si-SIRT1 3 after 48 hours post transfection. (C) Quantification data showing SIRT1 protein expression was significantly reduced with si-SIRT1 3 after 48 hours post transfection. Data represented as Mean± SD. n=3, NS: none significant. * P<0.05, **P<0.01, ****P<0.0001 versus control or si-NC by one-way Anova and unpaired T-test.

3.4. Achievement of SIRT1 overexpression in endothelial cells by plasmid transfection

To further determine whether overexpressing SIRT1 can protect against PET-induced damage in endothelial cells, SIRT1 was overexpressed by transfection. As a result, compared to control group with empty vehicles, SIRT1-transfected HUVECs increased SIRT1 gene expression by 23294.1% (P<0.01), and increased SIRT protein level by 136.642% (P<0.01), confirming SIRT1 overexpression at both gene and protein level (Figure 4A-C).

DOI: 10.54254/2753-8818/2025.AU30222

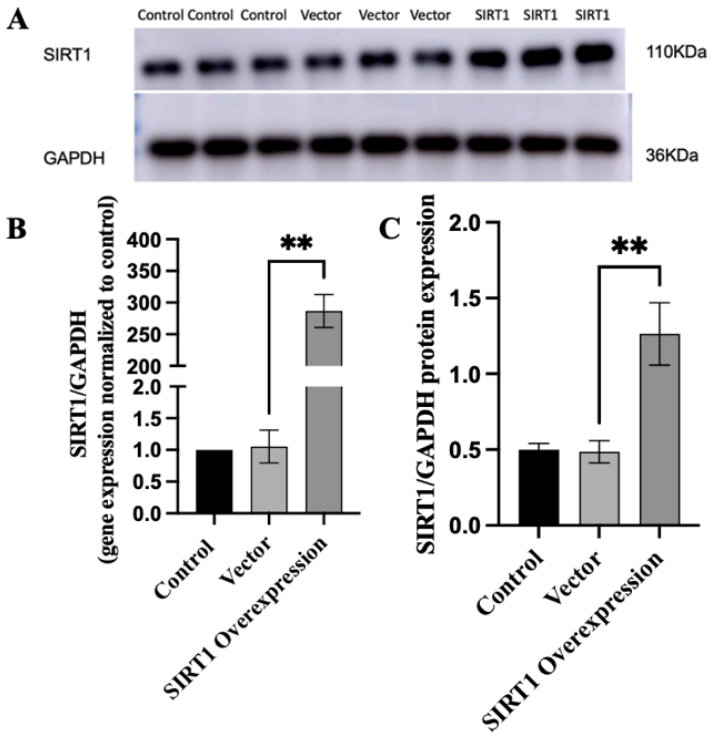

Figure 4. SIRT1 overexpression was achieved by transfecting endothelial cells

(A) SIRT1 gene expression was significantly up regulated with SIRT1 overexpression after 48 hours post transient transfection. (B) Representative images of Western Blot showing SIRT1 protein expression after 48 hours post transfection. (C) Quantification data showing SIRT1 protein expression was significantly increased with SIRT1 overexpression after 48 hours post transfection. Data represented as Mean± SD. n=3, NS: none significant. * P<0.05, **P<0.01 versus control by one-way Anova and unpaired T-test.

3.5. SIRT1's protection against PET-induced detrimental effects

To measure the effect of SIRT1 on PET-induced reduction of cell viability, HUVECs were treated with PET, with or without SIRT1 knockdown or overexpression. As a result, we observed that in comparison to the untreated control group, PET-treated HUVECs significantly reduced cell viability (ctrl: 100%, PET: 61.53%, P<0.0001), which was exacerbated by SIRT1 knockdown (SIRT1 siRNA: 45.38%%, P<0.0001, Figure 5A). In contrast, SIRT1 overexpression restored cell viability (95.08% P<0.0001, Figure 5A). Consistently, the cell apoptosis marker Caspase 3/7 was increased in PET-treated cells, which was enhanced by SIRT1 inhibition (Figure 5B). The apoptotic effect was reversed by SIRT1 overexpression. Taken together, the data indicated that SIRT1 was protective against PET-induced cell death.

To characterize the endothelial function in response to the PET insult, we measured eNOS levels (Figure 5C). PET treatment significantly suppressed eNOs production (ctrl: 0.27, PET: 0.19, P<0.0001). With SIRT1 knockdown, eNOS levels were further decreased in cells (SIRT1 siRNA: 0.06, P<0.0001), whereas SIRT1 overexpression greatly rescued the eNOS level (0.25, P<0.0001). The changes in eNOS indicated that PET effectively suppressed eNOs, which can be prevented by SIRT1.

DOI: 10.54254/2753-8818/2025.AU30222

We also measured ROS in the cells, which is another parameter reflecting cell stress (Figure 5D). We observed that PET increased ROS level compared to the control group (ctrl: 292640, PET: 383180, P<0.0001). The increase was enhanced with SIRT1 knockdown (SIRT1 siRNA: 785000, P<0.0001), which could be fully reduced by SIRT1 overexpression to the similar level in untreated control group (310113, P<0.0001). The results suggested that the increased ROS production may be an underlying factor causing cell apoptosis, which can be fully prevented by SIRT1.

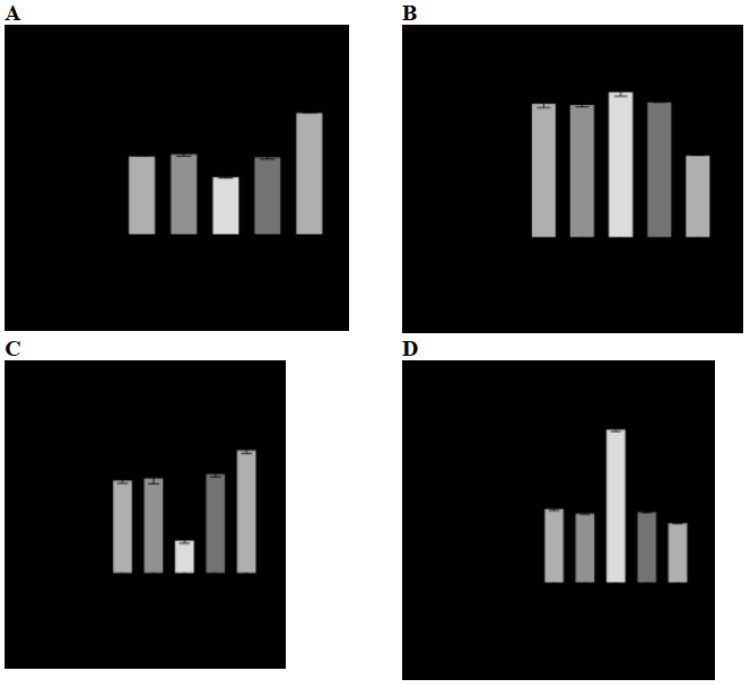

Figure 5. HUVEC cell function and viability with exposure to PET, SIRT1 inhibition, and SIRT1 overexpression

(A) HUVECs cell viability (normalized to control) under PET treatment and SIRT1 knockdown or overexpression. Data represented as Mean± SD. n=3, NS: none significant. * P<0.05, **P<0.01, ****P<0.0001 versus control by unpaired T-test. (B) HUVECs cell apoptosis under PET treatments and SIRT1 knockdown or overexpression. Data represented as Mean± SD. n=3, NS: none (C) HUVECs eNOS level under PET treatment and SIRT1 knockdown or overexpression. Data represented as Mean± SD. n=3, NS: none significant. ****P<0.0001 versus control by unpaired T-test. (D) HUVECs ROS level under PET treatment and SIRT1 knockdown or overexpression. Data represented as Mean± SD. n=3, NS: none significant. ****P<0.0001 versus control by unpaired T-test.

3.6. Transcriptomics analysis revealed diverse signaling pathways associated with PET cytotoxicity

Previous results demonstrated that PET imposed significant stress to endothelial cells, while SIRT1 downregulation exacerbated the cell damage. To better understand the molecular pathways underlying the cell damage, we performed RNA sequencing for control (NC), PET + NC treated, and PET+siSIRT1 treated HUVECs.

Principal component analysis displayed distinct gene expression profiles across the three groups (Figure 6A). PC1 demonstrates 88% of the variance, clearly separating NC from PET and siSIRT1 +

DOI: 10.54254/2753-8818/2025.AU30222

PET groups, which indicates PET's significant effect. PC2 analysis accounts for a 6% variance between PET and siSIRT1 + PET group. We selected the top 500 highly expressed genes to create a heatmap (Figure 6B). Many genes that were upregulated in the NC group were down regulated in the PET group. Furthermore, knocking down SIRT1 amplified the effect of PET, as shown by the further decreased gene expressions in the siSIRT1+PET group. Contrastingly, the genes that were upregulated in the PET group became further upregulated in the siSIRT1 + PET group.

To investigate differentially expressed genes (DEGs) involved in the endothelial injury process, we depicted volcano plots. In the comparison between PET and NC, we identified 2087 upregulated genes and 2533 downregulated genes (adjusted P value <0.05). In the volcano plot, we identified a few DEGs with significant fold changes that are associated with cardiovascular health (Figure 6C). HMGCS1 encoding enzymes catalyzing cholesterol biosynthesis was upregulated with PET treatment. The augmented expression of HMGCS1 may increase cholesterol level [3]. Enrichment analysis also showed that many lipid synthesis and metabolism pathways were upregulated (Figure 6D). Taken together, PET may increase the risk of atherosclerosis by disrupting lipid handling processes.

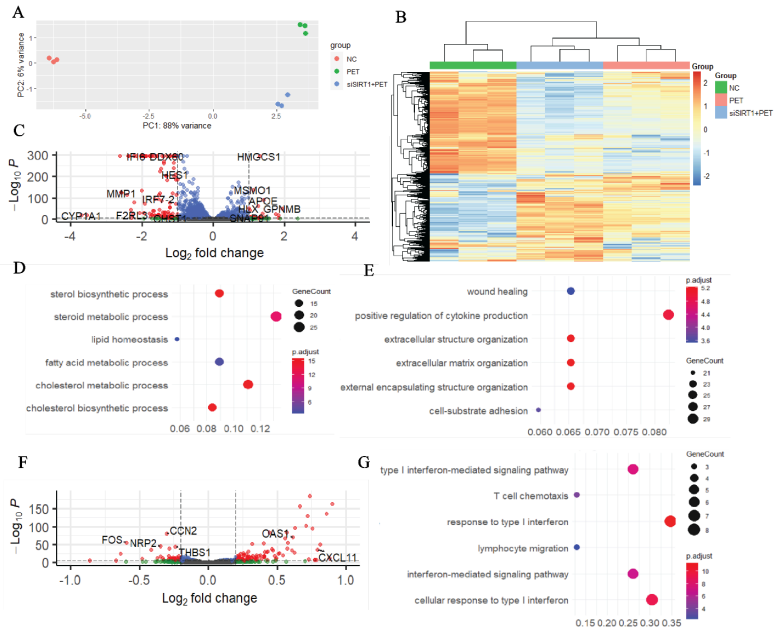

Figure 6. Transcriptomic profiling identifies key genes and pathways associated with endothelial damage

(A) Principal component analysis (PCA) revealed a clear separation between NC and PET-treated groups. (B) Heatmap of the top 500 highly expressed genes, clustered by unsupervised hierarchical analysis, identified three distinct clusters. (C) Volcano plot of PET vs. NC showing HMGCS1 upregulated, while PARP9, XAF1, HELZ2, THBS1, and SAMHD1 were downregulated. GO analysis of DEGs in PET vs. NC showing upregulated (D) and downregulated (E) pathways. (F) Volcano plot of siSIRT1 vs. PET showing HMGCS1 upregulated, while PARP9, XAF1, HELZ2, THBS1, and SAMHD1 were downregulated. (G) GO enrichment analysis of DEGs identified in siSIRT1 vs. PET highlighted strong upregulation of interferon-related pathways.

In downregulated genes, SAMHD1 is accountable for DNA repairs. The decrease in SAMHD1 expression may induce cell apoptosis and immune system dysfunction [6]. Similarly, PARP9 encodes a protein that can trigger the immune response and inflammation in cardiac fibrosis. The

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decreased expression of PARP9 may increase the chance of cardiac dysfunctions [7]. Some genes are related to endothelial function. Since THBS1 regulates nitric oxide signaling and proliferation in endothelial cells, its downregulation can lead to endothelial dysfunction and vessel impairment [8]. From GO enrichment analysis, we also identified some downregulated pathways controlling inflammation and tissue repair (Figure 6E). Additionally, HELZ2 gene encodes proteins that regulate lipid metabolism. The decreased expression of HELZ2 may cause more cholesterol and fats to accumulate in blood streams, possibly leading to cardiovascular diseases [9]. Interestingly, we identified XAF1, encoding proapoptotic factors that control cellular apoptosis. Down regulation of XAF1 might lead to less apoptosis, which might be a compensatory response to PET induced stress [10]. The decrease in pathways associated with tissue structural integrity indicated that PET may be a risk factor to weakened vascular junctions (Figure 6E).

In the comparison between siSIRT1 and PET, we identified 526 upregulated genes and 606 downregulated genes (adjusted P value < 0.05). In the volcano plot, we also identified a few DEGs that are associated with cardiovascular health (Figure 6F). CXCL11 encoding proteins that are responsible for inflammation response are upregulated. This can lead to prolonged inflammation response [11], which has been shown to recruit immune cells and impair vascular strength. The increase in OAS1 has been shown to promote monocyte adhesion, contributing to atherosclerosis [12]. Consistently, figure 6G demonstrated the activation of multiple immune pathways, some of which were interferon-mediated inflammation. Interferon signaling strongly blocks endothelial cell proliferation and growth, reduced NO production, and suppressed angiogenesis [13].

In the downregulated genes, the number of DEGs was limited. However, we still identified THBS1, which regulates NO production and thus vascular function [8]. NRP2, CCN2, and FOS are genes encoding proteins that are responsible for angiogenesis and cell growth [14]. Their decreased expression can limit endothelial self-repair and disrupt cell integrity [15].

4. Discussion

The toxicological profile of PET nanoplastics is alarming due to rising human exposure and their capacity to affect both the gastrointestinal and vascular systems. Recent evidence shows PET and other micro/nanoplastics in cardiovascular tissues, including thrombi and atherosclerotic plaques, with most particles measuring under 50 μm [3]. PET particles are not merely inert contaminants; their additives and breakdown products, such as antimony, are known to have endocrine-disrupting and potentially carcinogenic properties [16]. Chronic exposure to PET and its associated chemicals may exacerbate conditions such as inflammation, oxidative stress, and endothelial dysfunction, contributing to broader health risks such as cardiovascular disease and metabolic disorders.

This study demonstrated that PET impaired HUVEC integrity and function by significantly reduced endothelial cell viability, increased apoptosis, reduced eNOS levels and increased ROS concentrations, whereas SIRT1 protects against the PET-induced damage. Among the characteristics, eNOS is a critical enzyme for vascular homeostasis, producing nitric oxide (NO), which regulates vasodilation and reduces oxidative stress. A decrease in eNOS compromises these protective mechanisms, while elevated ROS levels promote oxidative stress and endothelial damage, potentially leading to vascular dysfunction. These findings are aligned with earlier studies reporting that exposure to PET suppressed cell proliferation, migration, and NO production in endothelial progenitor cells and endothelial cells [4]. Additionally, transcriptomics analysis revealed that PET significantly activated lipid metabolism and synthesis pathways, indicating its role in promoting atherosclerosis. Furthermore, genes regulating endothelial function and cell growth were strongly downregulated, which are consistent with the experiment findings in this study.

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By restoring a balance between pro-survival and pro-apoptotic signals, SIRT1 effectively maintains endothelial homeostasis in the presence of PET-induced stressors. Additionally, SIRT1 restored eNOS levels, a crucial factor for vascular health, by promoting nitric oxide (NO) production and counteracting oxidative stress. SIRT1 also reduced ROS levels, limiting oxidative damage and preserving cellular integrity. These observed effects are aligned with SIRT1's known functions as a regulator of endothelial function and a modulator of oxidative stress pathways. RNA sequencing analysis also proved the protective effects mediated by SIRT1. When SIRT1 was inhibited, pathways that are controlling endothelial cell junctions and function were significantly suppressed. Collectively, inflammation-related pathways were activated, imposing inflammatory stress on endothelial cells. Genes regulating cell growth and proliferation, such as THBS1 and FOS were downregulated, which suggests that SIRT1 is a crucial mediator to attenuate PET induced oxidative stress by protecting endothelial cells from inflammation.

Some limitations exist for this study. Given the detection of plastic concentrations as low as 1.62 μg/ml in human blood [2], future studies should explore the chronic effects of lower PET doses and investigate the potential for adaptation or recovery mechanisms, such as mitohormesis, in endothelial cells exposed to nanoplastics. Moreover, the interplay between PET particle properties (e.g., size, shape, surface charge) and their biological effects remains to be fully elucidated. Smaller particles, which penetrate deeper into tissues, may exacerbate ROS production and other harmful pathways. Investigating these variables will provide a more comprehensive understanding of the toxicological profile of PET nanoplastics and inform strategies to mitigate their impact on human health.

5. Conclusion

This research contributes to the growing understanding of the biological effects of nanoplastics, a ubiquitous and concerning environmental pollutant. For the first time, we discovered that PET reduced cell viability; increased cell apoptosis; decreased cell eNOS level; and increased cell ROS level. On the other hand, the experimental group which treated with SIRT1 overexpression showed that SIRT1 was able to mediate those negative effects. Through transcriptomics analysis, we found that SIRT1 mediated PET induced damage by maintaining endothelial homeostasis and suppressing inflammatory responses. In general, this study demonstrates the toxic effects of PET nano-plastics on endothelial cells and highlights the potential of SIRT1 as a protective factor. These findings, combined with evidence of widespread human exposure to microplastics, emphasize the urgent need for further research to elucidate their long-term health impacts. Exercising, or treatment with SIRT1 can revert and protect against the harmful effects of PET microplastics. As microplastics are a ubiquitous environmental pollutant, understanding their biological effects and developing mitigation strategies are critical for safeguarding public health and environmental sustainability.

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