Microplastics exposure of endometrial stromal cells (eSCs) *in vitro* leads to changes in proliferation and decidualization

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Background

Microplastics are degraded plastic particles less than 5 mm in diameter and are widespread in the environment.[1] Exposure to microplastics is frequent, if not daily, and they are primarily ingested, inhaled, or adsorbed.[2] The concentration of microplastics in human blood can vary, but prior research has indicated a range between 1 to 5 µg/mL.[3] Due to the proposed toxic nature of plastic particles, coupled with plastic use in food packaging, medicine, and other industries, it is important to investigate the potential effects of microplastics on the human body.[4]

One area of concern regarding the toxicological nature of microplastics is their effect on reproductive health and fertility.[5] Prior research suggests that conditions such as endometriosis are correlated with environmental exposures, although microplastics are under-researched in this context.[6] Therefore, we explored the potential effects of microplastics on female reproductive health and the uterine environment.

To investigate this, endometrial stromal cells from healthy controls enrolled in the **Research OutSmarts Endometriosis (ROSE) Study** were expanded in culture and exposed to a range of doses of microplastic particles. Cell proliferation and decidualization, a process of cell differentiation required for embryo implantation and pregnancy, were analyzed to understand significant changes.[7] Thus, our research question is: how does exposing human endometrial stromal cells (eSCs) to varying concentrations and sizes of microplastics influence cell proliferation and decidualization in both short-term and long-term cultures? We hypothesize that increased microplastics concentration *in vitro* will impair cell proliferation and decidualization at both time points.

Methods

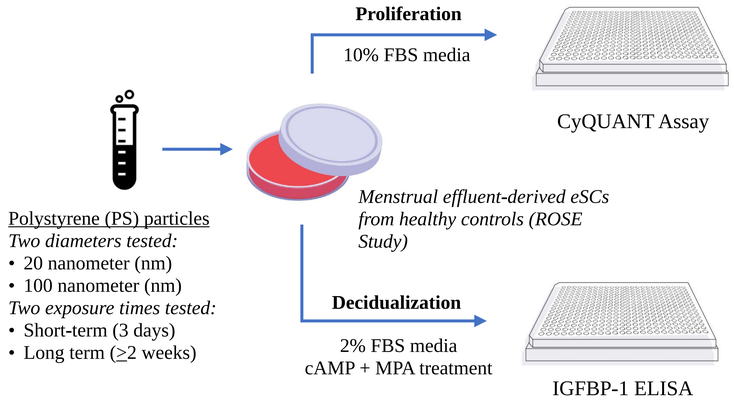

Figure 1. Cells were plated at 0.8-1.4x10^4 cells/mL *in vitro* and exposed to sonicated ThermoFisher Scientific 3000 Nanosphere polystyrene (PS) microplastics for 3 days or ≥2 weeks. Two diameters of PS were tested including 20 nm (Catalog # 3020A) and 100 nm (Catalog # 3100 A) particles and they were added in concentrations ranging from vehicle-treated to 200 µg/mL. Replicates were tested for proliferation and decidualization effects following maintenance in 10% FBS media and 2% FBS media, respectively. IGFBP-1 ELISA was used to determine the extent to which the cells decidualized following cAMP + MPA-induced treatment (0.5mM+10^-7M) with PS exposure. Comparatively, proliferation was analyzed using a CyQUANT assay.

Results

Proliferation

Short-term exposure (3 days)

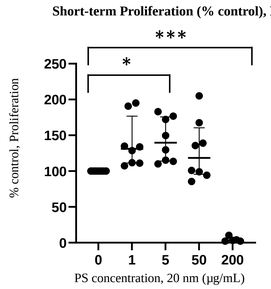

Figure 2. Displays the % control of relative cell number obtained from CyQUANT for eSCs exposed to PS 20 nm for 3 days. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.0002 and are therefore significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that 0 v. 5 µg/mL has an adjusted p-value of 0.0247 with slight (*) significance and 0 v. 200 µg/mL prove to be significant as well (***).

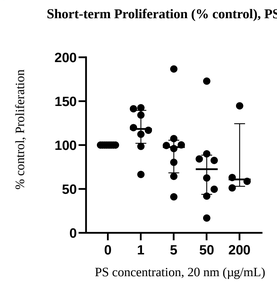

Figure 3. Displays the % control of relative cell number obtained from CyQUANT for eSCs exposed to PS 100 nm for 3 days. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.0424 and are therefore slightly significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that no significance was found between each group.

Long-term exposure (at least 2 weeks)

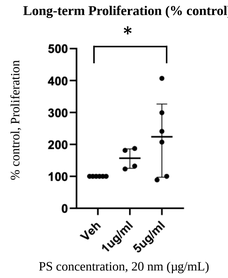

Figure 4. Displays the % control of relative cell number obtained from CyQUANT for eSCs exposed to PS 20 nm for at least 2 weeks. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.0205 and are therefore significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that vehicle-treated v. 5 µg/mL have an adjusted p-value of 0.0276 with slight (*) significance.

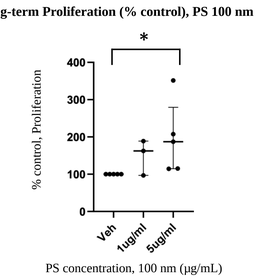

Figure 5. Displays the % control of relative cell number obtained from CyQUANT for eSCs exposed to PS 100 nm for at least 2 weeks. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.0348 and are therefore significant (p-value < 0.05.) A Dunn's multiple comparisons test (p-value < 0.05) reveals that vehicle-treated v. 5 µg/mL have an adjusted p-value of 0.0270 with slight (*) significance.

Decidualization

Short-term exposure (3 days)

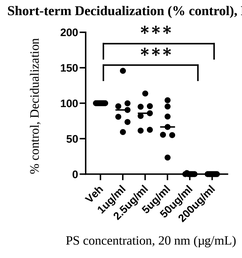

Figure 6. Displays the % control of IGFBP-1 obtained from ELISA assays for eSCs exposed to PS 20 nm for 3 days. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of <0.0001 and are therefore significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that both vehicle-treated v. 50 µg/mL and vehicle-treated v. 200 µg/mL have an adjusted p-value of 0.0001 with (***) significance.

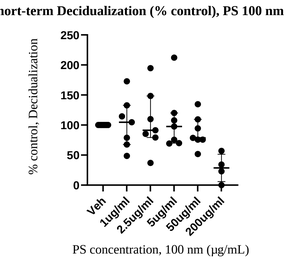

Figure 7. Displays the % control of IGFBP-1 obtained from ELISA assays for eSCs exposed to PS 100 nm for 3 days. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.8843 and are therefore not significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that no significance was found.

Long-term exposure (at least 2 weeks)

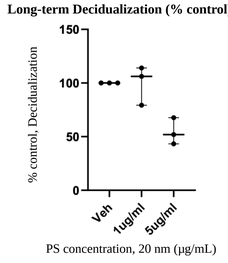

Figure 8. Displays the % control of IGFBP-1 obtained from ELISA assays for eSCs exposed to PS 20 nm for at least 2 weeks. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.0357 and are therefore significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that the data are not significant.

Need more data for significance
-in progress-

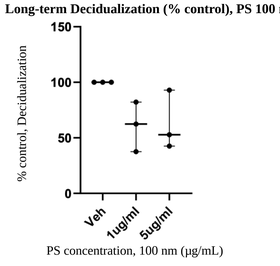

Figure 9. Displays the % control of IGFBP-1 obtained from ELISA assays for eSCs exposed to PS 100 nm for at least 2 weeks. Using a Kruskal-Wallis test (p-value < 0.05), the data have an overall p-value of 0.0679 and is therefore not significant. A Dunn's multiple comparisons test (p-value < 0.05) reveals that the data are not significant.

Conclusions

- Cell proliferation was affected more significantly when cells were exposed to PS 20 nm particles compared to PS 100 nm particles.
- At PS concentrations 1 µg/mL to 5 µg/mL, increased proliferative trends were observed compared to vehicle-treated cells in both short-term and long-term PS 20 nm treatment groups. Conversely, proliferation decreased significantly following 50 to 200 µg/mL exposure to 20 nm PS short-term. There was also an increase in proliferation between vehicle-treated and 5 µg/mL long-term PS exposure for 100 nm PS particles.
- Short-term exposure to PS 20 nm particles led to a significant decrease in decidualization of eSCs from vehicle-treated to 50 µg/mL and 200 µg/mL.
- Although not significant yet, long-term exposure to both PS 20 nm and PS 100 nm show downward trends in cell decidualization.

Future Steps

- Perform a **lactate dehydrogenase (LDH) assay** to further test for cell viability and cytotoxicity. This can provide more insight into the quality of the cells which are proliferating in culture.
- Run additional long-term proliferation and decidualization experiments to improve power of significant trends.
- Determine mechanisms of action: Run **western blots** to determine significant protein pathways (ex. PI3K/AKT, ERK, etc.) which may mediate PS-mediated effects on proliferation and decidualization. Alternatively, bulk RNA sequencing can be performed to identify gene expression changes induced by PS particles.
- Explore exposure effects with smaller diameter particles (ex. nanoplastics).

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