

Article

Impact of Degradation of Polyethylene Particles on Their Cytotoxicity

Yudai Ikuno ¹, Hirofumi Tsujino ^{1,2,*}, Yuya Haga ¹ , Haruyasu Asahara ¹ , Kazuma Higashisaka ^{1,3} and Yasuo Tsutsumi ^{1,4,*}

¹ Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

² The Museum of Osaka University, 1-20 Machikaneyamacho, Toyonaka, Osaka 560-0043, Japan

³ Institute for Advanced Co-Creation Studies, Osaka University, 1-1 Yamadaoka, Suita, Osaka 565-0871, Japan

⁴ Global Center for Medical Engineering and Informatics, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

* Correspondence: htsujino@phs.osaka-u.ac.jp (H.T.); ytsutsumi@phs.osaka-u.ac.jp (Y.T.)

Abstract: Microplastics are ubiquitous in the environment, including in the ocean, soil, and air. Therefore, there are concerns regarding human exposure. Since it is known that the surface of microplastics in various environments is chemically deteriorated by external factors such as ultraviolet rays and waves, it is essential to evaluate the biological effects of degraded microplastics. In this study, we experimented by accelerating the degradation of polyethylene (PE) using vacuum ultraviolet light and prepared PE samples with different degrees of degradation. Then, we evaluated the effects of undegraded and variously degraded PE on cells using cytotoxicity tests. Based on the cytotoxicity test results, we saw a tendency for increased cytotoxicity with increasing degradation. Therefore, this study substantially links the deterioration of microplastics with their biological effects.

Keywords: microplastics; degradation; cytotoxicity



Citation: Ikuno, Y.; Tsujino, H.; Haga, Y.; Asahara, H.; Higashisaka, K.; Tsutsumi, Y. Impact of Degradation of Polyethylene Particles on Their Cytotoxicity. *Microplastics* **2023**, *2*, 192–201. <https://doi.org/10.3390/microplastics2020015>

Academic Editor: Nicolas Kalogerakis

Received: 15 November 2022

Revised: 8 May 2023

Accepted: 9 May 2023

Published: 22 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Microplastics are particles ≤ 5 mm in size that are ubiquitous in the environment, including in the ocean [1,2], soil [3], and air [4], and there are concerns about their impact on the organisms living there and on humans through the food chain [5,6]. Fish are known to unintentionally ingest microplastics in water [7], and microplastics have been detected in edible fish [8,9] and shellfish [10]. In addition, they have been detected in the bodies of seabirds, turtles, and marine mammals [9]. Microplastics have also been detected in salt [11,12] and drinking water [13,14]. Furthermore, microplastics have been detected in the human placenta [15], lung tissue [16], and blood [17] and may accumulate in the human body. Since microplastics enter the human body, they may create associated health hazards. Therefore, assessing the impact of microplastics on the human body is an urgent issue [18]. In recent years, the effects of microplastics on human health have been clarified, and it has been shown that there is a correlation between fecal microplastics and inflammatory bowel disease [19]. There are various types of plastics, such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS). Due to the availability of standard particles, various in vivo and in vitro studies are often conducted using PS particles. In studies using 1, 4, and 10 μm PS, high concentrations of 1 μm PS particles were shown to be cytotoxic to the human intestinal epithelial cell line Caco-2, and their intracellular uptake was also confirmed. However, no histologically detectable lesions or inflammatory responses were observed upon oral administration of PS to mice [20]. In addition, long-term exposure to 40–100 μm PS particles has been reported to reduce fertility in male mice [21]. Thus, although various studies have been performed, further research is required to investigate the effect of microplastics on human health.

The effects of the size, shape, and surface properties of extracorporeal microparticles are often considered when assessing their biological impact [22,23]. In the environment, microplastics exist in a wide variety of types, shapes, and sizes [24], therefore, it is essential to consider these when evaluating their biological effects. Studies have been conducted to evaluate the effects of the composition and shape of microplastics. A study evaluating the uptake of PE, PP, polyethylene terephthalate (PET), and PVC into the human intestinal epithelial cell line Caco-2 reported that the highest uptake was of PE [25]. Further, the release of inflammatory cytokines has been reported to be higher in the presence of fragments than that in the presence of spherical particles [26]. Microplastics existing in the environment are known to deteriorate and weather due to various external factors [27]. Degradation processes include mechanical fragmentation, photodegradation, thermal degradation, and biodegradation. Microplastics are miniaturized and broken down into different sizes and shapes, such as by mechanical fragmentation. Moreover, due to photodegradation and thermal degradation, the surface of the microplastic is chemically degraded, and functional groups containing oxygen, such as carbonyl groups and hydroxy groups, are introduced to the surface. Furthermore, when physical forces such as waves and wind are applied to the surface of microplastics that have become brittle due to photodegradation, cracks and holes occur on the surface [27]. In this way, microplastics in the environment deteriorate due to various factors and acquire various physical properties not found in pure plastics. Among these, chemical modification of the surface of microplastics by carbonyl groups and hydroxyl groups generated by degradation is considered to be an important factor in evaluating the effects of microplastics on organisms. It has been reported that PS modified with carboxyl and amino groups exhibits stronger cytotoxicity than unmodified polystyrene [28]. The surface modifications associated with the deterioration of microplastics may have negative effects on living organisms. Therefore, when evaluating the biological effects of microplastics in the environment, it is also necessary to consider the effects of their degradation. Furthermore, it is thought that the degree of degradation of microplastics varies because microplastics in the environment are exposed to ultraviolet rays for different periods of time and at different temperatures. Therefore, in evaluating the biological effects of microplastics, it is necessary to consider various degradation states and evaluate the biological effects of the degree of degradation. However, few studies have considered this aspect of surface degradation, let alone the degree of deterioration. In order to evaluate the biological effects in consideration of the surface deterioration of microplastics, it is necessary to prepare samples that have the same physical properties such as size and surface morphology, but differ only in the chemical state of the surface.

Therefore, in this study, we prepared PE samples that were rapidly degraded by irradiation with vacuum ultraviolet light (VUV). Furthermore, we prepared PE samples with various degrees of degradation by changing the VUV irradiation time. Next, we evaluated the biological effects of PE degradation through cytotoxicity tests to determine if the cytotoxicity of PE increases as it degrades.

2. Materials and Methods

2.1. Preparation and Measurement of Surface Characteristics of Various Degraded PE Samples

In this study, flo-thene UF (UF-20S; Sumitomo Seika Chemicals Company, Osaka, Japan) was used as the PE particle sample. According to the vendor's information, the medium particle size was 24 μm . PE degradation experiments were performed using a FLAT EXCIMER EX-mini (Hamamatsu Photonics K.K., Shizuoka, Japan). This device irradiates VUV with a wavelength of 172 nm in the range of 86 \times 40 mm. First, the PE sample was spread on the bottom of a Petri dish and placed on a lab jack installed inside the device. Next, the lab jack was adjusted so that the distance between the PE sample and the light source was approximately 10 mm, and VUV irradiation was conducted for 0.5 h. After the irradiation, the PE sample was collected in a sample bottle using a packaging paper. The same operation was repeated thrice, and the three PE samples were collected in one sample bottle and used as one of the degraded PE samples. According to the above

preparation procedure, three degraded PE samples were prepared with irradiation times of 0.5, 1, and 2 h to prepare a total of nine degraded samples.

Attenuated total reflection infrared (ATR-IR) spectral measurements were performed using an infrared spectrometer (FT/IR-4700) (JASCO Corporation, Tokyo, Japan) equipped with a triglycine sulfate (TGS) detector. A diamond ATR crystal (incident angle of 45°; one reflection) fixed in the horizontal ATR accessory was used. All spectra were collected with 32 scans at a resolution of 4 1/cm in the range of 4000–500 1/cm wavenumber. First, a background spectrum without any sample on the ATR crystal was measured immediately before sample measurements. The PE sample was then placed on the ATR crystal and pressed onto it to record the IR spectrum. The force with which the PE sample was pressed against the ATR crystal was adjusted so that the maximum absorbance values for each sample were similar for each measurement. The raw spectra are presented as pATR ($-\log I/I_0$) spectra, where the sample spectral intensity (I) was divided by the background spectral intensity (I_0). The IR spectra of the undegraded PE and nine degraded PEs were measured.

2.2. Cell Lines and Cultures

The cell lines used were human alveolar adenocarcinoma cells (A549), which are lung epithelial cells potentially exposed to microplastics [16,29]. A549 cells were purchased from RIKEN Cell Bank (Ibaraki, Japan) and cultured in Dulbecco's modified Eagle's medium (high-glucose) (FUJIFILM Wako Pure Chemical, Osaka, Japan) supplemented with 10% fetal bovine serum (Biosera, Nuaille, France) and 1% (*v/v*) penicillin-streptomycin-amphotericin B suspension (FUJIFILM Wako Pure Chemical). The cells were cultivated at 37 °C and 5% CO₂ and passaged every 2–4 days. Passaging was performed by incubating with trypsin in 0.2 mM ethylenediamine tetra-acetic acid (EDTA) in phosphate-buffered saline (PBS) at 37 °C for 5 min after aspirating the culture medium and washing with EDTA-PBS. The reaction was stopped by adding culture medium and the cells were separated by centrifugation at 1000 rpm for 5 min. Finally, the supernatant was aspirated, dispersed in a culture medium, and the cells were seeded in a 100φ dish.

2.3. Cytotoxicity Assessment of PE

A549 cells were seeded in 96-well plates at a density of 1.0×10^4 cells per well and allowed to attach for 24 h. The cell culture medium was then replaced with 100 μL of cell culture medium containing 0.001% carboxymethyl cellulose sodium salt (CMC) (FUJIFILM Wako Pure Chemical) as a dispersant at different concentrations (0–80 g/L) of undegraded or degraded PE samples. Since undegraded PE is hydrophobic, its dispersibility in medium is low, whereas degraded PE has improved dispersibility in the medium owing to the introduction of a hydrophilic functional group. Therefore, CMC was used as a dispersant to reduce the influence of dispersibility differences. After 24 h of incubation, cell cytotoxicity was evaluated using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Tokyo Chemical Industry, Tokyo, Japan). Measurements were corrected for background signals by subtracting the values from wells incubated without cells and then relating them to the solvent control. Additionally, cell viability for each measurement was fitted with a sigmoid curve to calculate the 50% inhibitory concentration (IC₅₀) using Python.

2.4. Measurement of Particle Size of PE Samples

Particle size distribution measurements were outsourced to the Japan Laser Corporation (Tokyo, Japan). The device used for this study was a HELOS laser diffraction instrument (Sympatec GmbH, Clausthal-Zellerfeld, Germany), and the dry method was used for the measurements. The measurement range was 0.1–350 μm. The samples used were undegraded and degraded PE, and the degraded PE was newly prepared by VUV irradiation for 1 h. From the volume-based cumulative distribution (Q₃) (%) obtained from the measurement, the ratio (dQ₃) within each measurement section was calculated, with

the whole being 1. The volume-based frequency distribution (q_3^*) was calculated using the following equation:

$$q_3^* = dQ_3 / \log_{10}(X_u/X_o) \quad (1)$$

where, X_u represents the upper limit value of the particle size in the section, and X_o represents the lower limit value. dQ_3 represents the ratio within the measurement section, with the whole being 1.

2.5. Measurement of Surface Morphology of PE Samples

Field emission scanning electron microscopy (SU6600) (FE-SEM; Hitachi, Ltd., Tokyo, Japan) was performed to analyze the surface morphology of PE. The samples used were undegraded and degraded PE, and the degraded samples in particle size distribution measurements were used. The prepared undegraded and degraded PE were fixed on the sample table with carbon tape and an accelerating voltage of 20 kV was used for measurements.

3. Results and Discussion

3.1. Measurement of Surface Characteristics of Degraded PE Particles

Since microplastics in the environment deteriorate when exposed to UV rays for a long time, to reproduce this effect in a short period, we irradiated PE using high-energy VUV and produced degraded PE. Infrared absorption spectra were measured by ATR-IR to determine the state of PE surface degradation. Measurements were performed for the undegraded PE and nine degraded PE samples. Raw ATR-IR spectra of the undegraded PE sample and the representative degraded PE sample are shown in Figure 1. The peaks around 2915 1/cm and 2848 1/cm were due to the C–H asymmetric and symmetric stretching of PE, respectively. Another peak at approximately 1465 1/cm was due to the C–H scissor bending in PE. Additionally, the C=O stretching (1715 1/cm) and the –C–O– (1174 1/cm) peaks confirmed that carbonyl groups and esters were introduced into the PE sample through degradation. This is a general characteristic of plastic degradation, indicating that the PE sample was successfully chemically degraded using this method. It was also confirmed that carbonyl groups and esters were similarly introduced into the other eight degraded PE samples.

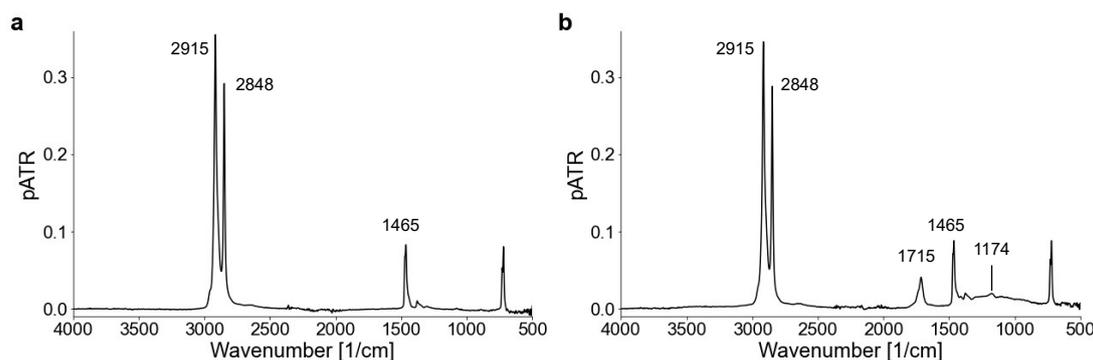


Figure 1. Representative raw ATR-IR ($pATR = -\log I/I_0$) spectra of the undegraded PE sample (a) and the degraded PE sample (b).

In ATR-IR, the peak height varies depending on the strength with which the sample is pressed against the ATR crystal; therefore, the ratio of the peak height of C=O (1715 1/cm) to that of C–H (1465 1/cm) ($C=O/C-H$) was used to determine the degree of degradation. The $C=O/C-H$ ratio was 0.000 (sample 1) before degradation. A degradation treatment of 0.5–2 h resulted in a degraded PE sample with a $C=O/C-H$ ratio of 0.156 (sample 2), 0.166 (sample 3), 0.177 (sample 4), 0.290 (sample 5), 0.310 (sample 6), 0.349 (sample 7), 0.464 (sample 8), 0.508 (sample 9), and 0.513 (sample 10). This indicates that the degree of deterioration progressed along with VUV irradiation time. It was thought that the difference

in the deterioration state of the PE samples treated with the same VUV irradiation time might have been due to the unevenness in spreading the PE samples on the Petri dish.

3.2. Cytotoxicity Assessment of PE

To evaluate the effects of the undegraded and degraded PE samples on cells, a cytotoxicity assessment using the MTT assay was performed. The cell viability curves based on the MTT assay for undegraded sample 1, slightly degraded sample 2, and highly degraded sample 8 are shown in Figure 2. The results demonstrated a decreased A549 cell viability only in the presence of degraded PE. No marked cytotoxicity was observed in the presence of undegraded PE at the tested concentration range. In addition, cells treated with highly degraded PE had lower viability than those treated with slightly degraded PE in the 10–40 g/L PE concentration range, suggesting that PE with a higher degree of degradation has a stronger effect on A549 cells.

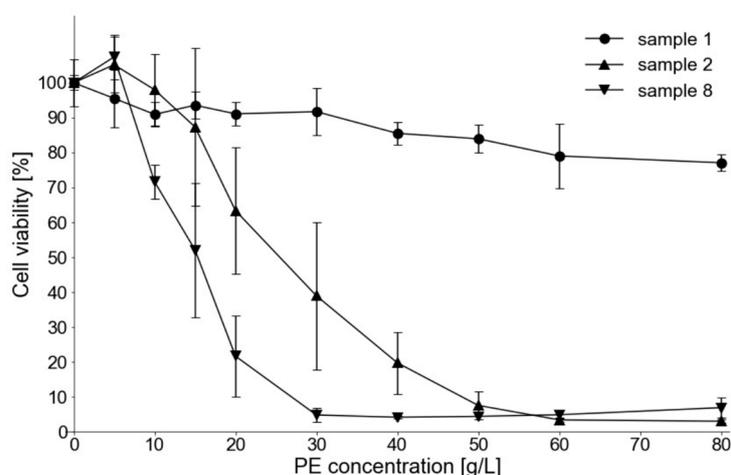


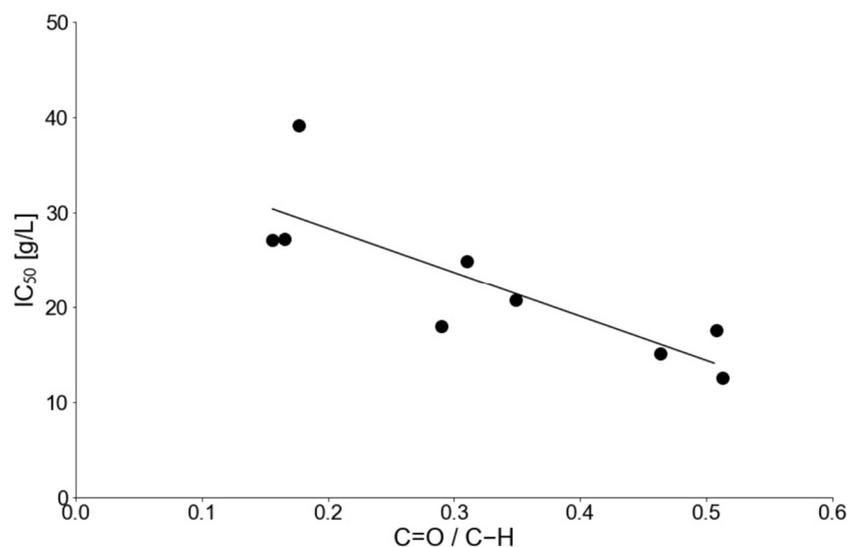
Figure 2. Cell viability curves obtained from MTT assay.

The IC_{50} values were determined to quantitatively evaluate the effects of the nine degraded PEs (Table 1). However, because the IC_{50} of undegraded PE could not be calculated, it was set to ≥ 80 . A smaller IC_{50} value indicates stronger cytotoxicity. In fact, the IC_{50} values of sample 2, with a degree of degradation of 0.156, and sample 8, with a degree of degradation of 0.464, were 27.1 and 15.1, respectively, (Figure 2) and the IC_{50} value of sample 8, which is considered to have strong cytotoxicity, was lower than that of sample 2. To clarify the relationship between the degree of degradation and IC_{50} , the obtained IC_{50} values were plotted against the degree of degradation ($C=O/C-H$) of the degraded PE (Figure 3). Cytotoxicity tended to increase as the degree of PE degradation increased. In this study, a linear relationship was found between the degree of degradation and IC_{50} within the range of the degree of degradation that was measured. The correlation coefficient was -0.824 , indicating that the degree of PE degradation and IC_{50} have a strong negative correlation. It is suggested that the cytotoxicity of degraded PE tends to increase depending on the degree of degradation.

Since the degree of degradation reflects the peak height of the carboxyl groups, it was presumed that cytotoxicity increased with the number of carboxyl groups. Nanoparticles with carboxyl-modified surfaces are more cytotoxic than unmodified nanoparticles, causing an increased cellular uptake and damage to cell membranes [28,30]. This suggests that the degraded PE exhibited cytotoxicity due to the formation of carboxyl groups on the surface during the degradation process, resulting in damage to the cell membrane. Furthermore, this suggested that increased carboxyl groups enhanced cytotoxicity. This important result demonstrated surface degradation dependence, which cannot be measured for simple surface-altered samples.

Table 1. Degree of degradation (C=O/C–H) and IC₅₀ value for each PE sample.

| Sample Number | C=O/C–H Ratio | IC ₅₀ [g/L] |
|---------------|---------------|------------------------|
| 1 | 0.000 | ≥80 |
| 2 | 0.156 | 27.1 |
| 3 | 0.166 | 27.2 |
| 4 | 0.177 | 39.1 |
| 5 | 0.290 | 18.0 |
| 6 | 0.310 | 24.9 |
| 7 | 0.349 | 20.8 |
| 8 | 0.464 | 15.1 |
| 9 | 0.508 | 17.6 |
| 10 | 0.513 | 12.6 |

**Figure 3.** IC₅₀ value for the degree of degradation (C=O/C–H).

According to a previous study that calculated the intake of microplastics from various foods and drinking water sources, the median daily intake for adults was 583 ng/capita/day [31]. Therefore, the concentration at which cytotoxicity occurred in this experiment was unrealistic, and there was a possibility of almost no effect on living organisms. However, the pharmacokinetics of microplastics in the body are not well understood, and they may accumulate in specific organs. Therefore, the possibility of exposure to high concentrations of microplastics cannot be ruled out. Moreover, the cytotoxicity test used for this study evaluates the most severe negative effect, i.e., cell death. Therefore, in the future, it is necessary to evaluate the effects on cells other than cell death, such as inflammatory cytokine production.

Considering the possibility of a change in particle size due to degradation, the particle size distributions of the undegraded and degraded PE samples were measured. A freshly prepared degraded PE sample with a degree of degradation of 0.342 was used. The results of the volume-based frequency distributions are shown in Figure 4a. The horizontal axis represents particle size (μm), and a logarithmic scale was used for ease of viewing. The medium particle size was found to be $24.19 \pm 0.02 \mu\text{m}$ for undegraded PE and $26.63 \pm 0.06 \mu\text{m}$ for degraded PEs. The measured value of the undegraded PE aligned with that disclosed by the distributor. Since VUV irradiation causing the particles to become larger is not feasible, the increase in the median particle size is likely due to agglomeration or particle loss during sample collection. Cytotoxicity is unlikely to be due to changes in particle size, as the size changes with PE degradation were small. Figure 4b shows an enlarged view of the 0.2–2 μm size range of the volume-based frequency distribution shown in Figure 4a. This confirmed the presence of PE particles with a size of less than

2 μm in both undegraded and degraded PE. In addition, no significant difference in particle size distribution was observed in this size region, suggesting that there is no change in particle size in a minute area, such as the generation of fine particles. Particles of this size could directly be taken up by the cells [32]. Therefore, it is conceivable that undegraded and degraded PE of this size were taken up by cells, and only degraded PE had adverse effects, such as lysosomal damage, in cells taken up. In addition, introducing functional groups to the surface of PE after degradation may change the route of uptake into cells and the uptake ability, and the increase in uptake may produce enhanced cytotoxicity. In the future, it is necessary to evaluate the effect of uptake on cytotoxicity by measuring the uptake capacity and evaluating the uptake route.

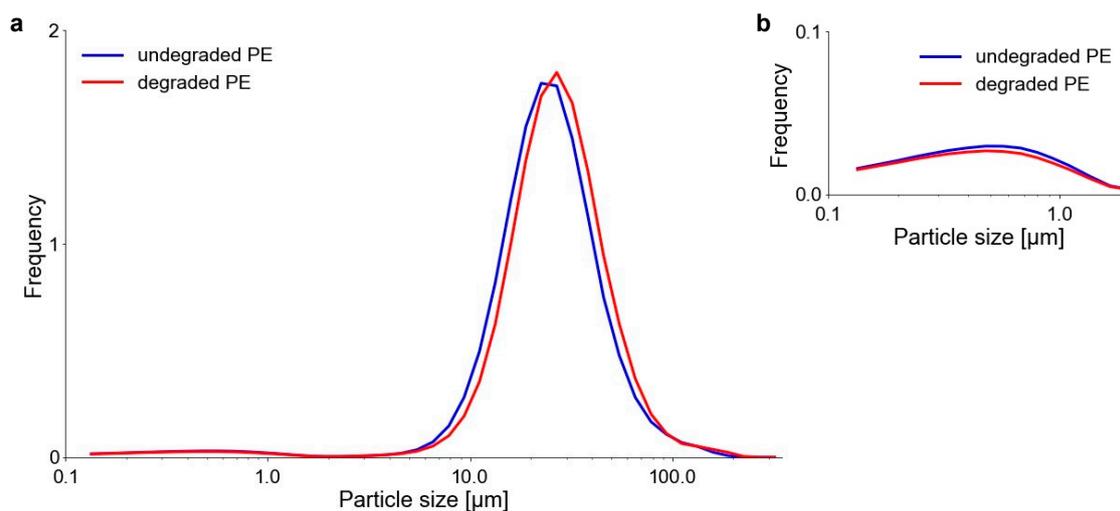


Figure 4. Volume-based frequency distribution of undegraded and degraded PE (a) and its enlarged view in the 0.1–2 μm range (b).

To evaluate the morphological changes on the PE surface during VUV degradation, FE-SEM observation was conducted (Figure 5). Degraded PE with the same degree of degradation as the particle size distribution measurement was used. It was observed that both the undegraded and degraded PE were elliptical with irregular unevenness, and not spherical. No significant change in morphology, including sharpening of the shape due to the degradation, was confirmed, and the shape remained elliptical. In addition, no significant changes in surface morphology, such as cracking or pitting, were observed. This indicates that the surface morphology of the deteriorated PE prepared in this study does not change, and that only the chemical state of the surface is different. This suggested that the cytotoxicity of degraded PE was not due to changes in morphology but due to changes in surface functional groups. However, it is known that the surface of microplastics present in the environment becomes brittle due to deterioration, resulting in cracks and holes on the surface [27]. Therefore, it is necessary to evaluate what biological effects microplastics with such surface morphology exhibit and whether there is a synergistic effect with the chemical state of the microplastic surface. For that purpose, a standard sample that simulates the surface morphology of microplastics in the environment is required. This may be achieved by applying a mechanical fragmentation process to the degraded PE prepared in this experiment.

Additionally, the IC_{50} of undegraded PE was expected to be 37.7 based on the intercept of the approximate straight line. However, the IC_{50} of undegraded PE cannot be determined from the results and is considered to be ≥ 80 g/L. Therefore, the relationship between the degree of degradation of PE and IC_{50} is not a simple linear relationship. In other words, the cytotoxicity of the PE particles greatly increased with degradation and gradually increased depending on the degree of degradation.

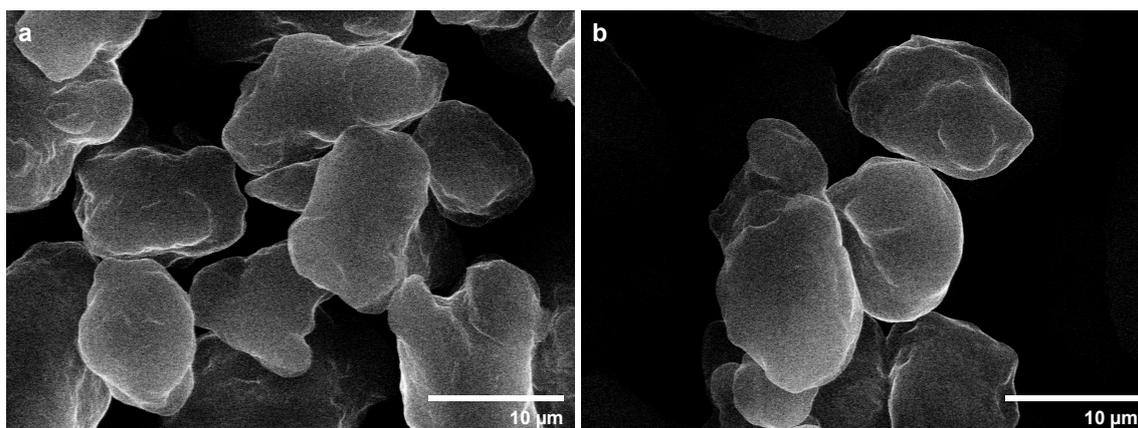


Figure 5. Surface morphology of undegraded PE (a) and degraded PE (b). Scale bars: 10 µm.

Microplastics in the environment show not only various states of deterioration, but also have various types, sizes, and shapes. Furthermore, it is known that the difference in their physical properties has different effects on living organisms. For example, in studies using 1, 4, and 10 µm PS, high concentrations of 1 µm, PS particles were shown to be cytotoxic to the human intestinal epithelial cell line Caco-2 [20]. A study evaluating uptake into the human intestinal epithelial cell line Caco-2 using PE, PP, PET, and PVC reported that the highest uptake of PE [25]. Furthermore, the release of inflammatory cytokines has been reported to be higher in the presence of fragments than in the presence of spherical particles [26]. Considering these facts and the results of this research, it is thought that an evaluation that integrates the effects of degradation and other physical properties will be necessary in the future. The PE sample used in this study had a broad particle size distribution centering on 24 µm; therefore, the effect of particle size could not be evaluated. It is necessary to evaluate the effects of degradation and size by conducting evaluations using PE samples with uniform particle sizes. In addition, the VUV degradation method used in this study is also applicable to other plastic particles, such as PP and PS. Therefore, by preparing degraded samples of other plastic particles and evaluating their effects on the living body, it is thought that the relationship between degradation and types can be clarified. By conducting such evaluations and verifying the possibility of synergistic effects due to various physical properties, we believe that we can further deepen our understanding of the biological effects of microplastics in the environment.

Based on these findings, we suggest that PE microplastics may have a greater impact on living bodies, depending on the degree of degradation. Therefore, it is necessary to evaluate the degradation of microplastics in environmental distribution surveys and pharmacokinetics. However, there is almost no research focusing on the degradation of microplastics at present. The results of this study demonstrate the importance of degradation in microplastic risk analysis, and further research is expected.

4. Conclusions

In this study, we focused on the chemical degradation of the surface of microplastics, and conducted cytotoxicity tests to determine the effects on living organisms. Since the state of degradation of microplastics in the environment is thought to vary, PE particles were degraded using VUV to prepare various PE samples with different degrees of degradation. Additionally, the ratio of the peak height of C=O (1715 1/cm) to that of C–H (1465 1/cm) (C=O/C–H) obtained by ATR-IR spectra was used to determine the degree of degradation. The cytotoxicity test revealed that the PE particles exhibited cytotoxicity following degradation. The IC₅₀ obtained in the cytotoxicity test correlates well with the degree of PE sample degradation, suggesting that the cytotoxicity of the PE particles increases as the degree of degradation increases. In addition, it was confirmed that the fabricated degraded PE did not change in size or surface morphology, and only the chemical state of the PE surface

was different. This suggested that the cytotoxicity of degraded PE was not due to changes in size or morphology but due to changes in surface functional groups. This result is a very important as it demonstrates not only the influence of microplastic degradation but also the relationship between the degree of degradation and the cytotoxicity. This study contributes substantially to our understanding of the link between microplastic deterioration and its biological effects, and to the development of microplastic research that takes degradation into account.

Author Contributions: Y.I.: Writing, Data curation, Investigation, Methodology, Visualization; H.T. Conceptualization, Project administration; Y.H.: Methodology, Project administration; H.A.: Methodology; K.H.: Methodology; and Y.T.: Project administration, Supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Japan Science and Technology Agency, grant number JPMJSP2138, and Japan Society for the Promotion of Science, grant number JP21387863, and Ministry of Health Labour and Welfare, grant number JPMH21447937, and The Sumitomo Foundation for Environmental Research Projects.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Not Applicable.

Acknowledgments: The authors express their gratitude to Kei Ohkubo and Yuki Itabashi from the Institute for Open and Transdisciplinary Research Initiatives, Osaka University, Osaka, Japan, for the opportunity to use the FLAT EXCIMER EX-mini.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as Contaminants in the Marine Environment: A Review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [[CrossRef](#)] [[PubMed](#)]
2. Cincinelli, A.; Martellini, T.; Guerranti, C.; Scopetani, C.; Chelazzi, D.; Giarrizzo, T. A Potpourri of Microplastics in the Sea Surface and Water Column of the Mediterranean Sea. *Trends Anal. Chem.* **2019**, *110*, 321–326. [[CrossRef](#)]
3. Yang, L.; Zhang, Y.; Kang, S.; Wang, Z.; Wu, C. Microplastics in Soil: A Review on Methods, Occurrence, Sources, and Potential Risk. *Sci. Total Environ.* **2021**, *780*, 146546. [[CrossRef](#)]
4. Wright, S.L.; Ulke, J.; Font, A.; Chan, K.L.A.; Kelly, F.J. Atmospheric Microplastic Deposition in an Urban Environment and an Evaluation of Transport. *Environ. Int.* **2020**, *136*, 105411. [[CrossRef](#)] [[PubMed](#)]
5. Besseling, E.; Redondo-Hasselerharm, P.; Foekema, E.M.; Koelmans, A.A. Quantifying Ecological Risks of Aquatic Micro- and Nanoplastic. *Crit. Rev. Environ. Sci. Technol.* **2019**, *49*, 32–80. [[CrossRef](#)]
6. Presence of Microplastics and Nanoplastics in Food, with Particular Focus on Seafood. *EFSA J.* **2016**, *14*, 4501. [[CrossRef](#)]
7. Li, B.; Liang, W.; Liu, Q.-X.; Fu, S.; Ma, C.; Chen, Q.; Su, L.; Craig, N.J.; Shi, H. Fish Ingest Microplastics Unintentionally. *Environ. Sci. Technol.* **2021**, *55*, 10471–10479. [[CrossRef](#)]
8. Yagi, M.; Kobayashi, T.; Maruyama, Y.; Hoshina, S.; Masumi, S.; Aizawa, I.; Uchida, J.; Kinoshita, T.; Yamawaki, N.; Aoshima, T.; et al. Microplastic Pollution of Commercial Fishes from Coastal and Offshore Waters in Southwestern Japan. *Mar. Pollut. Bull.* **2022**, *174*, 113304. [[CrossRef](#)]
9. Ugwu, K.; Herrera, A.; Gómez, M. Microplastics in Marine Biota: A Review. *Mar. Pollut. Bull.* **2021**, *169*, 112540. [[CrossRef](#)]
10. Van Cauwenberghe, L.; Janssen, C.R. Microplastics in Bivalves Cultured for Human Consumption. *Environ. Pollut.* **2014**, *193*, 65–70. [[CrossRef](#)]
11. Yang, D.; Shi, H.; Li, L.; Li, J.; Jabeen, K.; Kolandhasamy, P. Microplastic Pollution in Table Salts from China. *Environ. Sci. Technol.* **2015**, *49*, 13622–13627. [[CrossRef](#)] [[PubMed](#)]
12. Peixoto, D.; Pinheiro, C.; Amorim, J.; Oliva-Teles, L.; Guilhermino, L.; Vieira, M.N. Microplastic Pollution in Commercial Salt for Human Consumption: A Review. *Estuar. Coast. Shelf. Sci.* **2019**, *219*, 161–168. [[CrossRef](#)]
13. Koelmans, A.A.; Mohamed Nor, N.H.; Hermsen, E.; Kooi, M.; Mintenig, S.M.; de France, J. Microplastics in Freshwaters and Drinking Water: Critical Review and Assessment of Data Quality. *Water Res.* **2019**, *155*, 410–422. [[CrossRef](#)] [[PubMed](#)]
14. Eerkes-Medrano, D.; Leslie, H.A.; Quinn, B. Microplastics in Drinking Water: A Review and Assessment. *Curr. Opin. Environ. Sci. Health* **2019**, *7*, 69–75. [[CrossRef](#)]
15. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Papa, F.; Rongioletti, M.C.A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First Evidence of Microplastics in Human Placenta. *Environ. Int.* **2021**, *146*, 106274. [[CrossRef](#)]

16. Amato-Lourenço, L.F.; Carvalho-Oliveira, R.; Júnior, G.R.; dos Santos Galvão, L.; Ando, R.A.; Mauad, T. Presence of Airborne Microplastics in Human Lung Tissue. *J. Hazard Mater.* **2021**, *416*, 126124. [[CrossRef](#)]
17. Leslie, H.A.; van Velzen, M.J.M.; Brandsma, S.H.; Vethaak, A.D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and Quantification of Plastic Particle Pollution in Human Blood. *Environ. Int.* **2022**, *163*, 107199. [[CrossRef](#)]
18. Wagner, S.; Reemtsma, T. Things We Know and Don't Know about Nanoplastic in the Environment. *Nat. Nanotechnol.* **2019**, *14*, 300–301. [[CrossRef](#)]
19. Yan, Z.; Liu, Y.; Zhang, T.; Zhang, F.; Ren, H.; Zhang, Y. Analysis of Microplastics in Human Feces Reveals a Correlation between Fecal Microplastics and Inflammatory Bowel Disease Status. *Environ. Sci. Technol.* **2022**, *56*, 414–421. [[CrossRef](#)]
20. Stock, V.; Böhmert, L.; Lisicki, E.; Block, R.; Cara-Carmona, J.; Pack, L.K.; Selb, R.; Lichtenstein, D.; Voss, L.; Henderson, C.J.; et al. Uptake and Effects of Orally Ingested Polystyrene Microplastic Particles In Vitro and In Vivo. *Arch. Toxicol.* **2019**, *93*, 1817–1833. [[CrossRef](#)]
21. Deng, Y.; Chen, H.; Huang, Y.; Wang, Q.; Chen, W.; Chen, D. Polystyrene Microplastics Affect the Reproductive Performance of Male Mice and Lipid Homeostasis in Their Offspring. *Environ. Sci. Technol. Lett.* **2022**, *9*, 752–757. [[CrossRef](#)]
22. Lu, F.; Wu, S.H.; Hung, Y.; Mou, C.Y. Size Effect on Cell Uptake in Well-Suspended, Uniform Mesoporous Silica Nanoparticles. *Small* **2009**, *5*, 1408–1413. [[CrossRef](#)] [[PubMed](#)]
23. Huang, X.; Teng, X.; Chen, D.; Tang, F.; He, J. The Effect of the Shape of Mesoporous Silica Nanoparticles on Cellular Uptake and Cell Function. *Biomaterials* **2010**, *31*, 438–448. [[CrossRef](#)]
24. Zhang, Y.; Kang, S.; Allen, S.; Allen, D.; Gao, T.; Sillanpää, M. Atmospheric Microplastics: A Review on Current Status and Perspectives. *Earth. Sci. Rev.* **2020**, *203*, 103118. [[CrossRef](#)]
25. Stock, V.; Laurisch, C.; Franke, J.; Dönmez, M.H.; Voss, L.; Böhmert, L.; Braeuning, A.; Sieg, H. Uptake and Cellular Effects of PE, PP, PET and PVC Microplastic Particles. *Toxicol. In Vitro* **2021**, *70*, 105021. [[CrossRef](#)] [[PubMed](#)]
26. Choi, D.; Hwang, J.; Bang, J.; Han, S.; Kim, T.; Oh, Y.; Hwang, Y.; Choi, J.; Hong, J. In Vitro Toxicity from a Physical Perspective of Polyethylene Microplastics Based on Statistical Curvature Change Analysis. *Sci. Total Environ.* **2021**, *752*, 142242. [[CrossRef](#)]
27. Duan, J.; Bolan, N.; Li, Y.; Ding, S.; Atugoda, T.; Vithanage, M.; Sarkar, B.; Tsang, D.C.W.; Kirkham, M.B. Weathering of Microplastics and Interaction with Other Coexisting Constituents in Terrestrial and Aquatic Environments. *Water Res.* **2021**, *196*, 117011. [[CrossRef](#)] [[PubMed](#)]
28. Shi, X.; Wang, X.; Huang, R.; Tang, C.; Hu, C.; Ning, P.; Wang, F. Cytotoxicity and Genotoxicity of Polystyrene Micro- and Nanoplastics with Different Size and Surface Modification in A549 Cells. *Int. J. Nanomed.* **2022**, *17*, 4509–4523. [[CrossRef](#)]
29. Prata, J.C.; da Costa, J.P.; Lopes, I.; Duarte, A.C.; Rocha-Santos, T. Environmental Exposure to Microplastics: An Overview on Possible Human Health Effects. *Sci. Total Environ.* **2020**, *702*, 134455. [[CrossRef](#)]
30. Petushkov, A.; Intra, J.; Graham, J.B.; Larsen, S.C.; Salem, A.K. Effect of Crystal Size and Surface Functionalization on the Cytotoxicity of Silicalite-1 Nanoparticles. *Chem. Res. Toxicol.* **2009**, *22*, 1359–1368. [[CrossRef](#)]
31. Mohamed Nor, N.H.; Kooi, M.; Diepens, N.J.; Koelmans, A.A. Lifetime Accumulation of Microplastic in Children and Adults. *Environ. Sci. Technol.* **2021**, *55*, 5084–5096. [[CrossRef](#)] [[PubMed](#)]
32. Mao, Z.; Zhou, X.; Gao, C. Influence of Structure and Properties of Colloidal Biomaterials on Cellular Uptake and Cell Functions. *Biomater. Sci.* **2013**, *1*, 896–911. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.