Supporting information

Green fabrication of PHBV Microbeads Using Dimethyl Isosorbide Solvent for Skin Exfoliatiors

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Experimental

Thermal performance.

Thermogravimetric analysis (TGA). Thermal stability of the samples was performed using a TGA-4000 instrument (PerkinElmer, USA). The sample weight was approximately 5 mg. The experiments were conducted under nitrogen flow with heating of the samples from 50 °C to 500 °C at a rate of 10 °C/min. The initial decomposition temperatures were determined at 10 % weight loss. The maximum degradation rate temperature was measured at the peak maximum in the DTG curve.

Differential scanning calorimetry (DSC). Thermal behaviors of the samples were investigated using DSC-8000 (Perkin-Elmer, USA) under nitrogen flow. The temperature and heat flow were calibrated with indium. Samples of 5 mg were analyzed over a temperature range from -50 °C to 200 °C at heating and cooling rates of 10 °C/min. The results were recorded from the first cooling scan and the second heating scan.

Residue determination of Polysorbate 80.

Previous studies showed large doses of polysorbate 80 have potential toxicity. Therefore the residue of polysorbate 80 was assessed. The residue of Polysorbate 80 in PHBV microbeads was determined using a UV-Vis-NIR spectrometer (CARY5000, US).[1,2] Polysorbate 80 aqueous solution was scanned in the wavelength range from 200-800 nm to determine the wavelength of maximum absorbance (at 232 nm). The absorbance was detected with a series concentration of polysorbate 80 aqueous solution at a wavelength of 232 nm and a standard concentration curve was prepared.

0.01 g of PHBV microbeads were soaked in 2 mL deionized water at 90 °C and mechanically stirred for 24 hours. Then the suspension was filtered with a 0.22 micron filter membrane, and the supernatant obtained was used as a sample for measurement of UV absorbance. Finally, the polysorbate 80 content was calculated based on the standard curve.

Residue determination of Dimethyl Isosorbide (DMI)

Residue determination of Dimethyl Isosorbide in PHBV microbeads was carried out by gas chromatography (8890, US). 0.02 g of PHBV microbeads was dissolved in 2 mL of dichloromethane and quantified with a flame ionization detector using capillary HP-5 (30 M× 0.320 mm×0.25 μm) column (Agilent technology) and Nitrogen as a carrier gas. It was

determined by a programmed temperature increase method. The initial temperature was 80 °C and heated to 260 °C at 15 °C/min for 10 min. The flow rate was 1.0 mL/min. 0.0015 g of DIM dissolved in 10 mL of dichloromethane acted as a reference. The residual solvent content was calculated with the help of a known concentration of DMI.

The concentration of residue DMI in PHBV microbeads in dichloromethane was calculated using equation (1) as follows:

$$\text{Concentration of residue DMI} = \frac{\text{Peak area of DMI in PHBV microbeads}}{\text{Peak area of DMI}} \times \text{Concentration of DMI} \quad (1)$$

The residue content of DMI in PHBV microbeads was calculated using equation (2) as follows:

$$\text{Residue content of DMI (\%)} = \frac{\text{Concentration of residue DMI in dichloromethane}}{\text{Concentration of PHBV microbeads in dichloromethane}} \times 100 \quad (2)$$

Phytotoxicity characterizations of second microplastics

In order to further discuss the effects of PHBV microbead degradation products such as secondary microplastics or nanoplastics on plant germination, secondary microplastics of PHBV microbeads were obtained by physical and mechanical degradation[3,4]. PHBV microbeads were suspended in deionized water at 90 °C and stirred constantly for 24 hours (heat degradation) to obtain a second microplastic suspension. Another method to gain the secondary microplastics was by mechanical grinding using Onyx mortar. The morphology of secondary microplastics of PHBV microbeads was characterized by SEM. The final suspension or the grinding fragments dispersed with deionized water (0 mg/L, 100 mg/L, 1000 mg/L) were applied to assess lettuce germination toxicity.

Supplementary Table and Figures

Preparation of PHBV microbeads

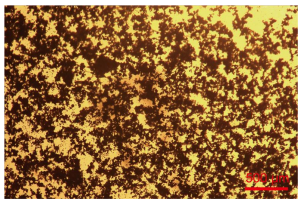

Figure S1. The photograph of optical microscope about PHBV microbeads prepared at the concentration of 3 wt% under 500 rpm, 80 °C.

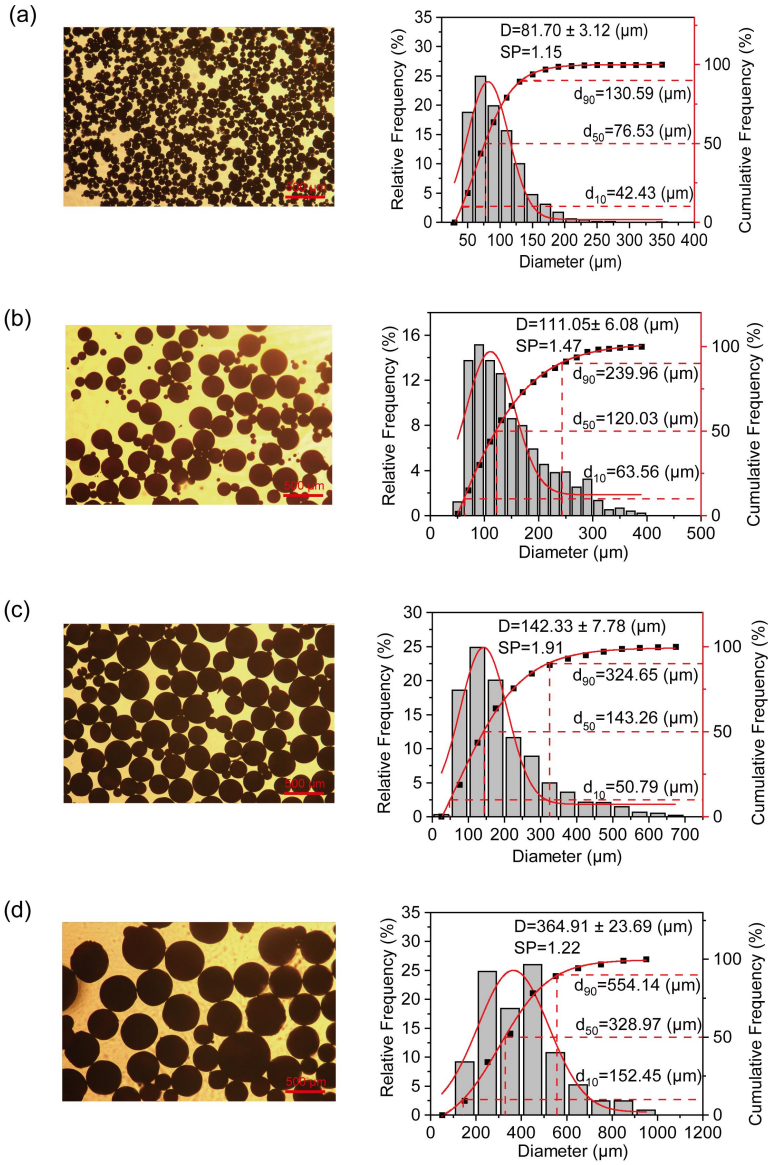

Figure S2. The photograph of optical microscope and size distribution about PHBV microbeads prepared at different concentration under 500 rpm, 80 °C. (a)-(e) represented the concentration at 5, 9, 12, and 15 wt%, respectively.

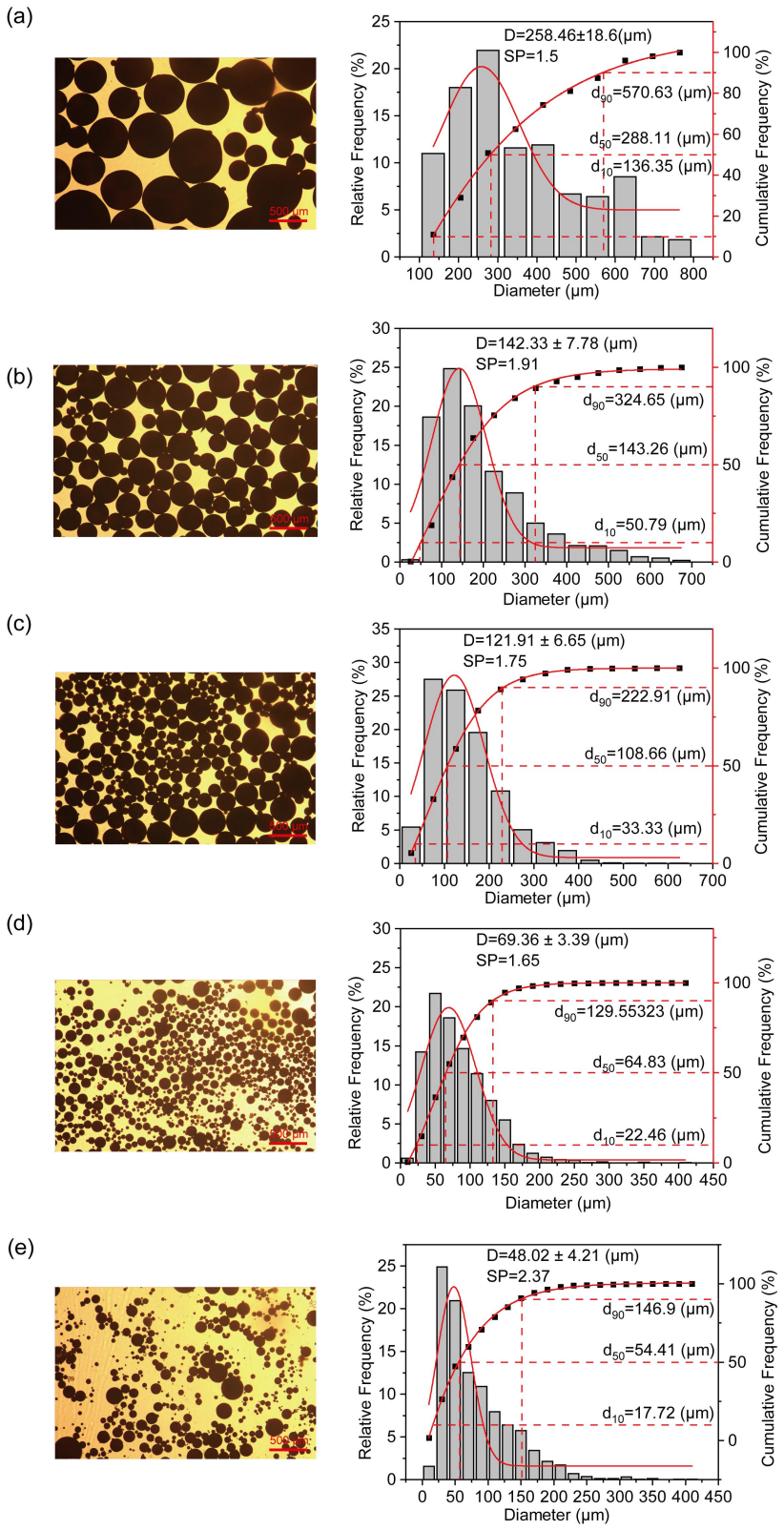

Figure S3. The photograph of optical microscope and size distribution about PHBV microbeads prepared at different stirring speed under 12 wt% of polymer solution, 80 °C. (a)-(e) represented the stirring speed at 300 rpm, 500 rpm, 700 rpm, 900 rpm, and 1200 rpm, respectively.

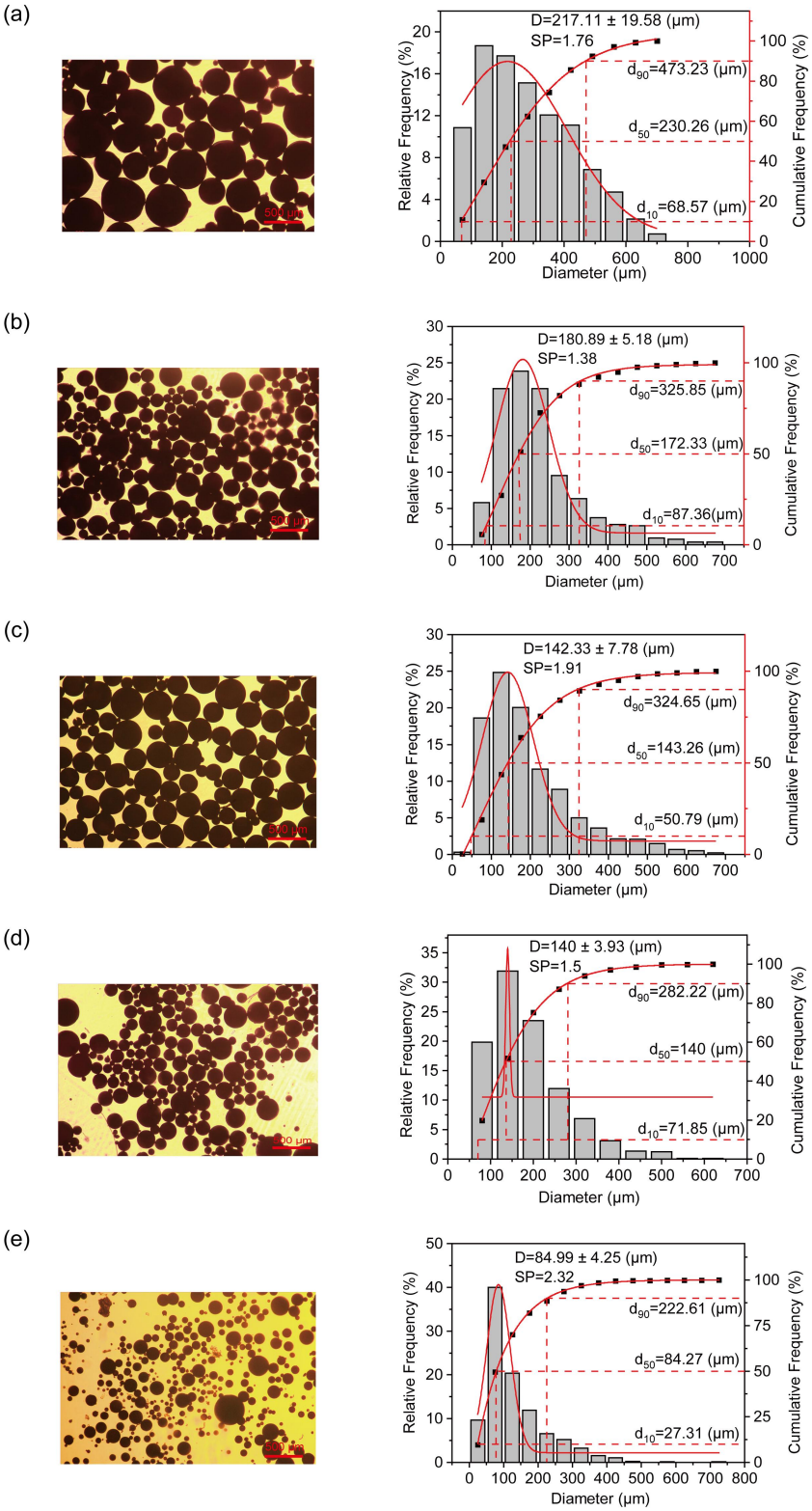

Figure S4. The photograph of optical microscope and size distribution about PHBV microbeads prepared at different concentration of surfactant under 12 wt% of polymer solution, 500 rpm, 80 °C. (a) - (d) represented the concentration of surfactant at 0.2, 0.5, 1, 2, and 4 wt%, respectively.

Thermal performance.

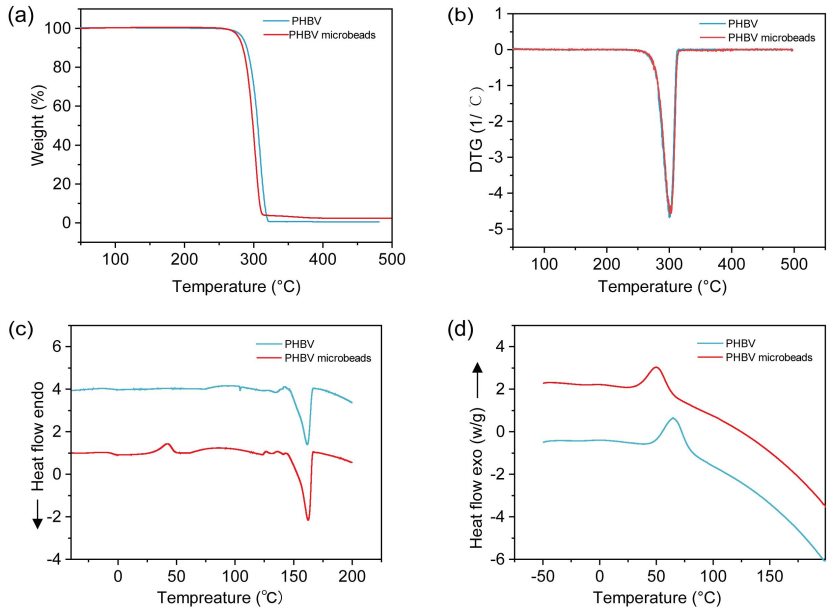

Figure S5. Thermal performance characterization of PHBV and PHBV microbeads. (a-d) refered to spectra of TGA, DTG, DSC of the second heating scan and the cooling scan, respectively.

Table 1. Thermal performance of PHBV and PHBV microbeads from TGA and DSC.

sample	$T_{10\%}$ (°C)	T_{max} (°C)	T_g (°C)	T_m (°C)	T_c (°C)
PHBV	285	300	-5.3	162	65
PHBV microbeads	284	301	-4.4	162	50

Residue determination of Polysorbate 80.

The standard curve of polysorbate 80 aqueous solution was shown as in Figure S6**.** The absorbance of the sample was 0.1092 and the concentration of residual polysorbate 80 was 9.0298 μg/mL. The content of polysorbate 80 in microbeads was about 0.18 %. Among the drugs already approved by the FDA (from Inactive Ingredient Search for Approved Drug Products), polysorbate 80 has different limits in different dosage forms. The limit of polysorbate 80 content per unit dose is roughly in the range of 0.02~5% w/v.[5] In Chinese Pharmacopoeia 2020 Part III, the content of polysorbate 80 required in the injection preparation of protein is not higher than 100 ug/mL.[6] In the National Food Safety Standard for Uses of Food Additives, polysorbate 80 is used for food additives with a content of 0.05~5 g/kg.[7] Recently, the National Health Commission of China also accepted the use of polysorbate 80 in food additives. In the safety and technical management specifications for cosmetics, no limit requirements for polysorbate 80 were found. Therefore, the polysorbate 80 content in PHBV microbeads was very low and PHBV microbeads prepared in this work were safe.

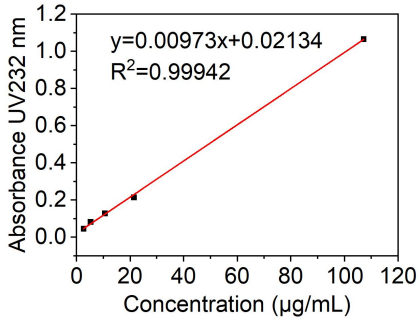

Figure S6. The standard curve of polysorbate 80 aqueous solution at 232 nm by UV-Vis-NIR spectrophotometry.

Residue determination of Dimethyl Isosorbide (DMI)

The gas **c**hromatograms of DMI and PHBV microbeads dissolved in dichloromethane was shown in Figure S7. The retention time of dichloromethane was at 2.6 min. The retention time of DMI was at 7.2 min and the peak area was about 62 pA·s. The peak area in PHBV microbeads at 7.2 min was about 24.6 pA·s. Thus, the concentration of residue DMI in dichloromethane was about 59.5 μg/mL. The residue content of DMI in PHBV microbeads was about 0.595 %. Though there was a very small residue, DMI as a bio-based green solvent was usually applied in cosmetics. Therefore, the PHBV microbeads prepared in this study are green and safe.

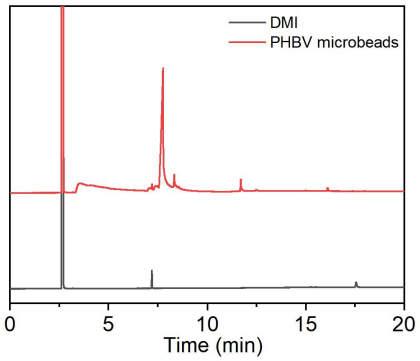

Figure S7. Gas **c**hromatograms of DMI and PHBV microbeads dissolved in dichloromethane.

Phytotoxicity characterizations

The size range of secondary microplastics about PHBV microbeads obtained from heat degradation was 30 nm to 40 μm and from mechanical grinding was 30 μm to 300 μm. (Figure S8). Secondary microplastics obtained by both ways had no significant influence on the germination rate of lettuce (Figure S9b). There was a slight promoting effect on root length at the microplastic concentration of 1000 mg/L. The secondary microplastics from mechanical grinding exhibited a positive impact on the lettuce germination index. All results indicate that PHBV microbeads and their physical degradation products were friendly to plants.

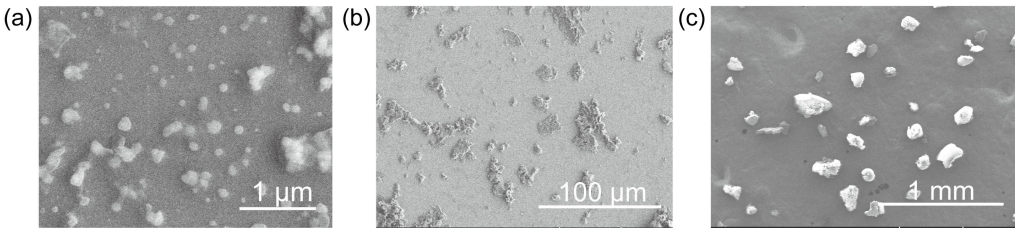

Figure S8. The SEM image about secondary microplastics of PHBV microbeads. (a-b) Heat degradation (b) Mechanical grinding.

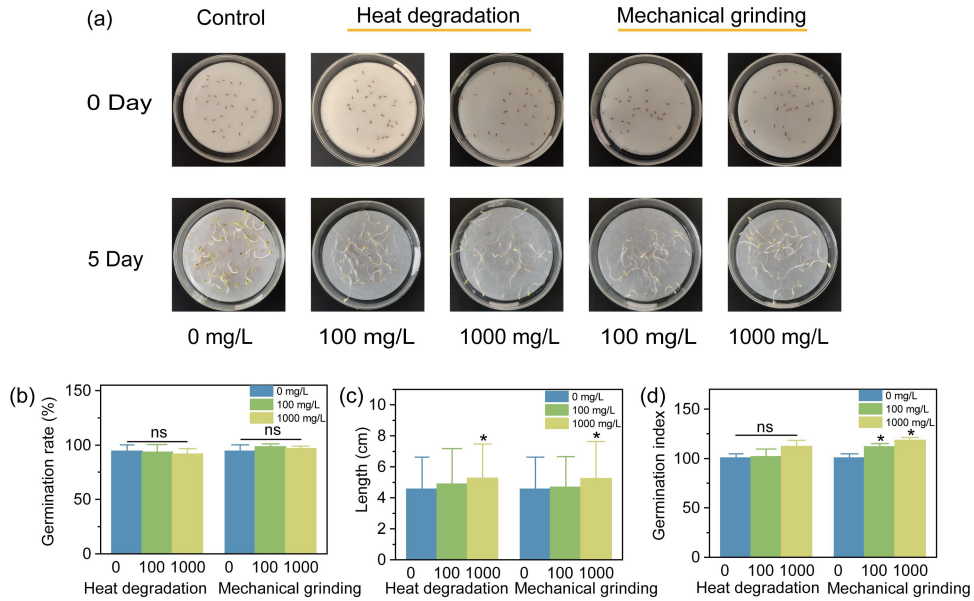

Figure S9. Effects about secondary microplastics of PHBV microbeads on lettuce germination. (a) Lettuce germination photograph treated with different concentration (0mg/L, 100 mg/L, and 1000 mg/L) of PHBV secondary microplastics from heat degradation and mechanical grinding for 5 days. (b)-(c) Germination rate, length and germination index treated with different concentration of secondary microplastics for 5 days, respectively. Statistical analysis was performed using a one-way ANOVA with the Turkey's test. ns indicates no significant difference among experimental groups. * indicates a significant difference compared to control ($P< 0.05$). Error bars represent the standard error.

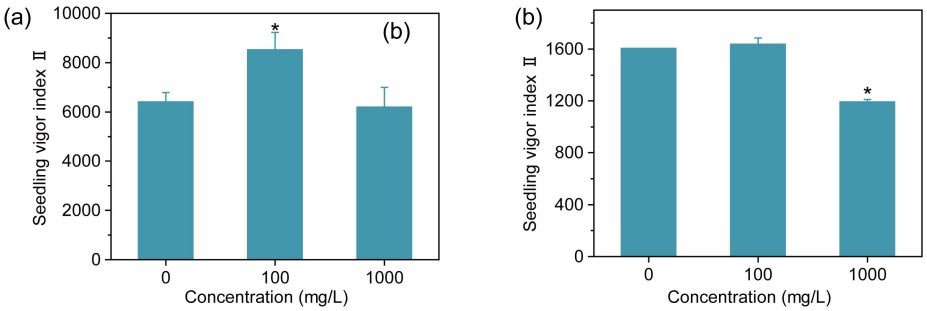

Figure S10. Phytotoxicity study of PHBV microbeads. (a) Seedling vigor index II of wheat treated with different concentration of PHBV microbeads (0, 100, and 1000 mg/L). (b) Seedling vigor index II of lettuce treated with different concentration of PHBV microbeads (0, 100, and 1000 mg/L). Statistical analysis was performed using a one-way ANOVA with the Turkey's test. * indicates a significant

difference compared to control (P< 0.05). Error bars represent the standard error.

Cleaning performance.

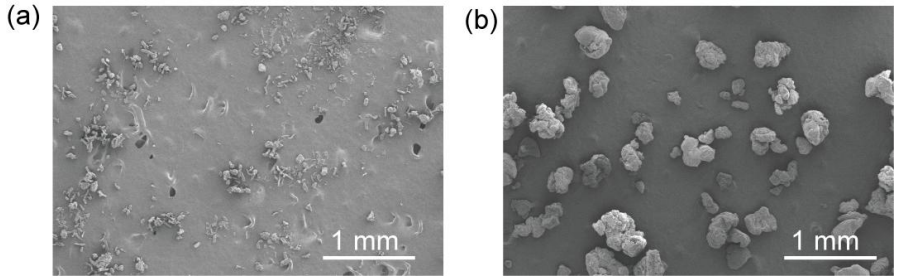

Figure S11. The SEM image of (a) MCC particles and (b) PE particles.

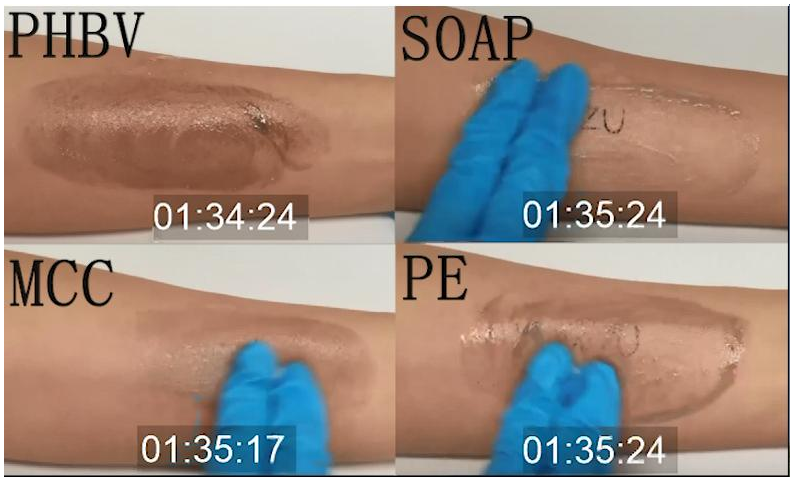

Figure S12. The cleaning image from cleansing movie when the contaminant was completely removed by PHBV microbeads.

References:

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