Electronic Supplementary Information

Biocompatible UV-absorbing Polymer Nanoparticles Prepared by Electron Irradiation for Application in Sunscreen

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Experimental

Materials

Tetramethylsilane as the reference material and 99.96% deuterated $CDCl_3$ as the solvent for NMR measurements were obtained from BK instruments, Republic of Korea. Dulbecco's phosphate-buffered saline (DPBS) with $MgCl_2$ and $CaCl_2$, Dulbecco's modified Eagle's medium (DMEM), antibiotic-antimycotic, newborn calf serum (NBCS), phosphate buffer saline (PBS) with pH of 7.4, and 1×Earle's balanced salt solution were purchased from Gibco, USA. dimethyl sulfoxide was purchased from Amresco, USA and BALB/3T3 clone A31 was purchased from American Type Culture Collection, USA. Neutral red and chlorpromazine were purchased from Sigma-Aldrich, USA. The most commonly used sunscreen active ingredients were obtained for comparative study: octisalate, oxybenzone, avobenzone, octocrylene, homosalate, octinoxate (Sigma-Aldrich, USA), TiO_2 rutile (50 nm), and ZnO nanoparticles (NPs) (80 - 200 nm) (RND Korea Co., Republic of Korea). The S2 UVA reference standard was obtained from Solar Light, USA.

3T3 Neutral Red Uptake Phototoxicity Test

All procedures for testing for phototoxicity of polymer NPs were conducted in accordance with the OECD TG 432. BALB/3T3 clone A31 was cultured in DMEM supplemented with 10% NBCS and 1% antibiotic-antimycotic (10 units/mL, 100 µg/mL Streptomycin, 0.25 µg /mL Amphotericin) at 37 °C and 5% CO_2 atmospheric content in an incubator. After incubation, two 96-well plates in the absence and presence of UVA illumination (–Irr and +Irr) were seeded with BALB/3T3 cells with a concentration of 1×10^4 cells per well. After another incubation period of 24 hours, the cells were exposed to 100 µL polymer NPs diluted in DPBS at various concentrations, 0.39, 0.78 1.56, 3.13, 6.25, 12.5, 25, and 50 µg/mL, for an hour in the incubator.

One plate was then placed under UVA (352 nm) illumination until it received the highest non-cytotoxic irradiation dose of 5 J m^{-2} using a Solar simulator (ATLAS, Germany). The other plate remained untouched by UV light. After 40 minutes, the time required to deliver the highest non-cytotoxic irradiation dose, the diluted polymer NPs were removed and the cells were carefully washed with 150 µL of PBS and were incubated once again for 22 hours. Next, 100 µL of a neutral red medium was added to the cells and the cells were further incubated for 3 hours in this state. After completing this incubation period, the cells were washed with 150 µL of PBS, and 150 µL of neutral red extract (water: ethanol: acetic acid = 49: 50: 1) was added to each well and then stirred thoroughly for 10 min. The absorbance of the extract at a 540 nm wavelength was measured six times independently using a Microplate Spectrophotometer (BioTek, USA).

Determination of the Phototoxic Potentials

The photo irritation factor (PIF) and the mean photo effect (MPE) values are determined using Phototox 2.0 software (ZEBET, Germany) by employing the following equations:

$$PIF = \frac{IC_{50}(-Irr)}{IC_{50}(+Irr)} \tag{1}$$

$$MPE = \frac{\sum_{i=1}^{n}\left[w_i \times \{(R_c(-Irr) - R_c(+Irr)\} \times \left|\frac{C/C^* - 1}{C/C^* + 1}\right|\right]}{\sum_{i=1}^{n} w_i} \tag{2}$$

where IC_{50} is the half maximal inhibitory concentration, w_i is the highest response value, R_c is the dose-response at concentration (C), and C^* is the equivalent concentration at which the +Irr response equals the –Irr response at C.

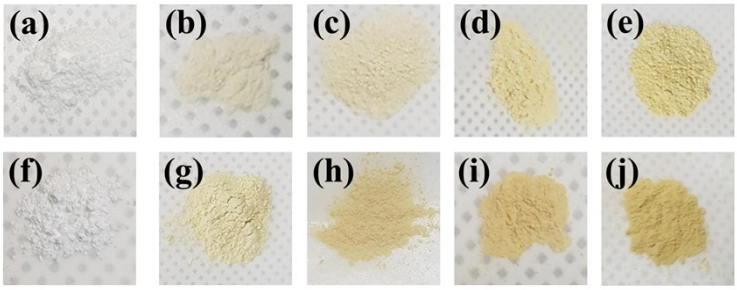

Fig. S1 Images of PMMA NPs irradiated with fluences of a) 0, b) 0.3, c) 0.6, d) 1.2, and e) 1.8 × 10^{17} cm^{-2} and PS NPs irradiated with fluences of f) 0, g) 0.3, h) 0.6, i) 1.2, and j) 1.8 × 10^{17} cm^{-2}. Yellowing of the polymers with increasing irradiation fluence is observed due to increasing absorption rate of blue light.

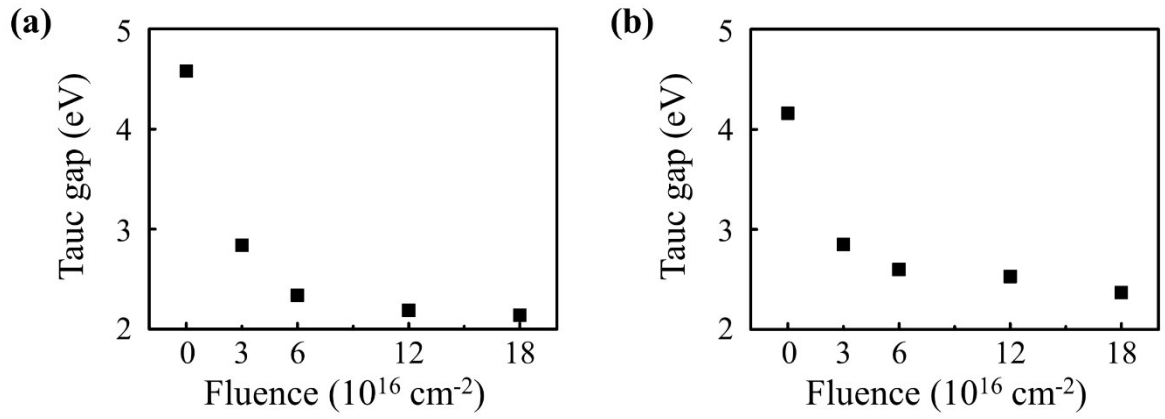

Fig. S2 Optical bandgap of a) PMMA and b) PS NPs at various electron fluences.

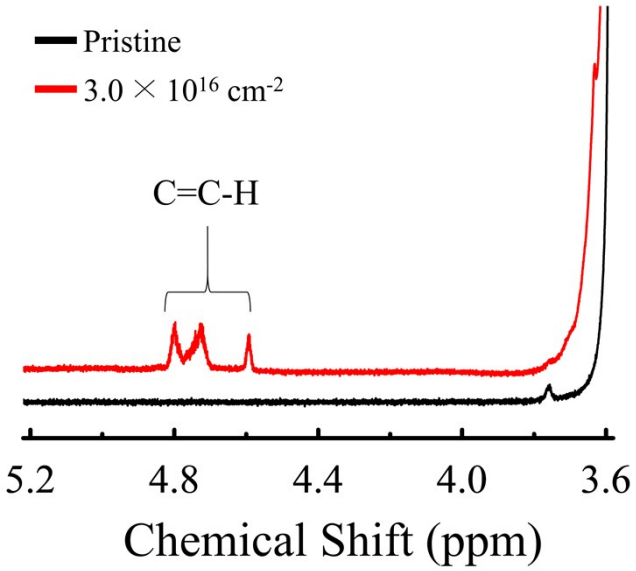

Fig. S3 ^{1}H NMR spectra of PMMA. Peaks located between 4.6 and 4.9 ppm of the spectrum plotted in red indicate the formation of aliphatic C=C bonds from electron irradiation.

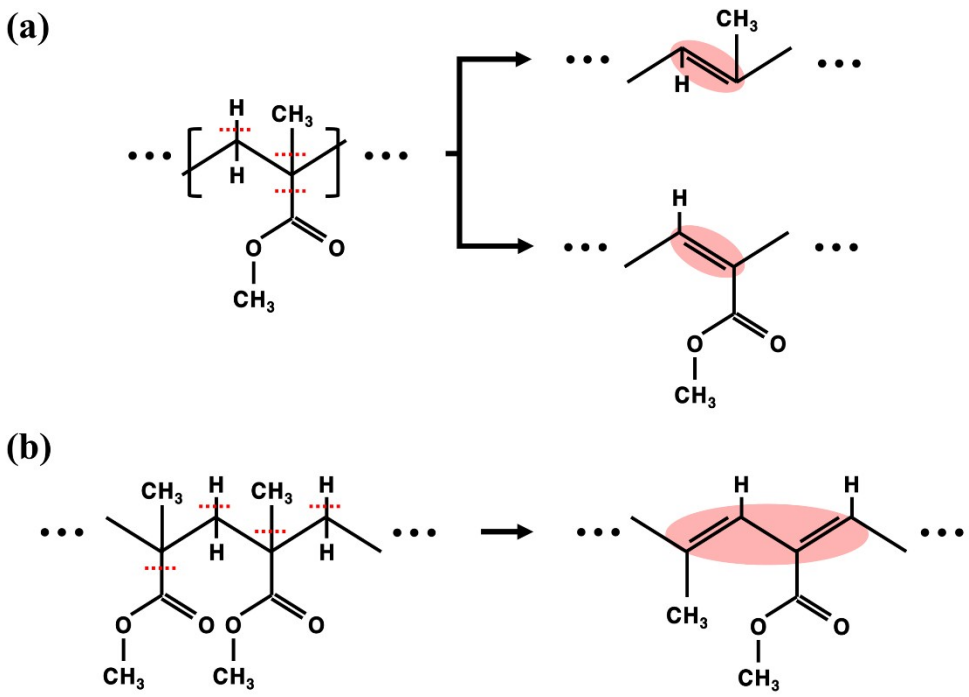

Fig. S4 Schematic illustration of the formation of (a) aliphatic C=C bond and (b) conjugated aliphatic C=C bonds by electron irradiation of PMMA.

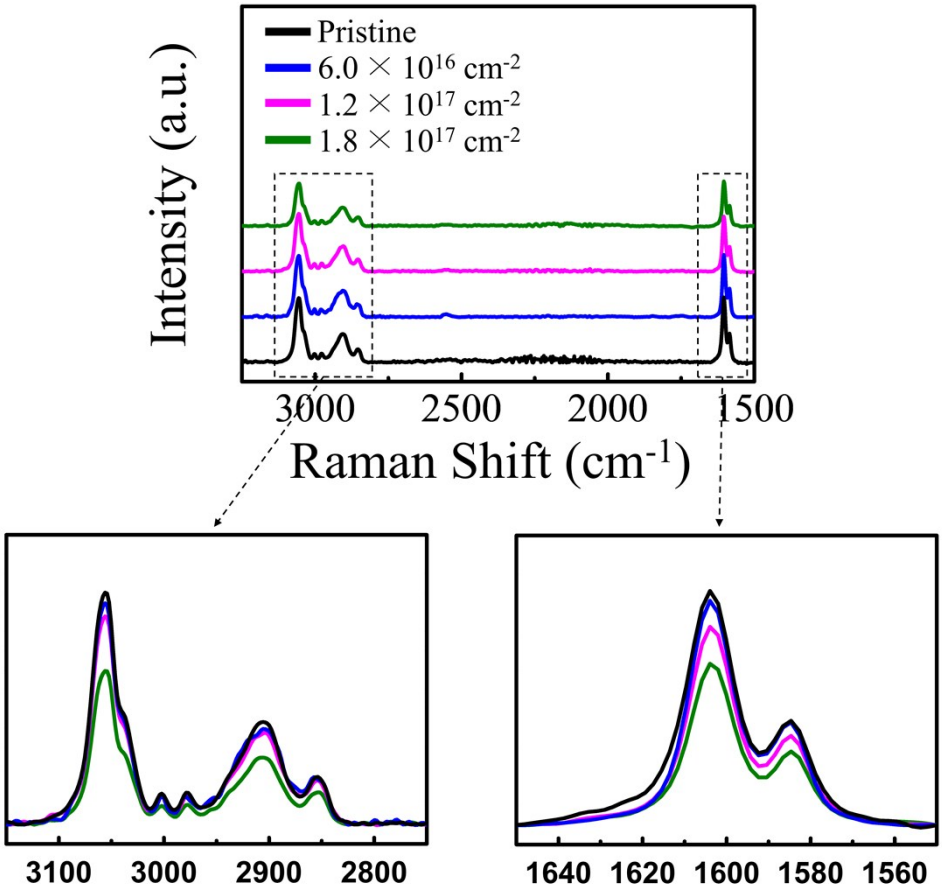

Fig. S5 FT-Raman spectra of pristine and electron-irradiated PS at various electron fluences. Peaks corresponding to aromatic C-H at 3054 cm^{-1}, aliphatic C-H at 2851 and 2902 cm^{-1}, and aromatic C=C vibrations at 1604 cm^{-1} reduce with increasing electron fluence.

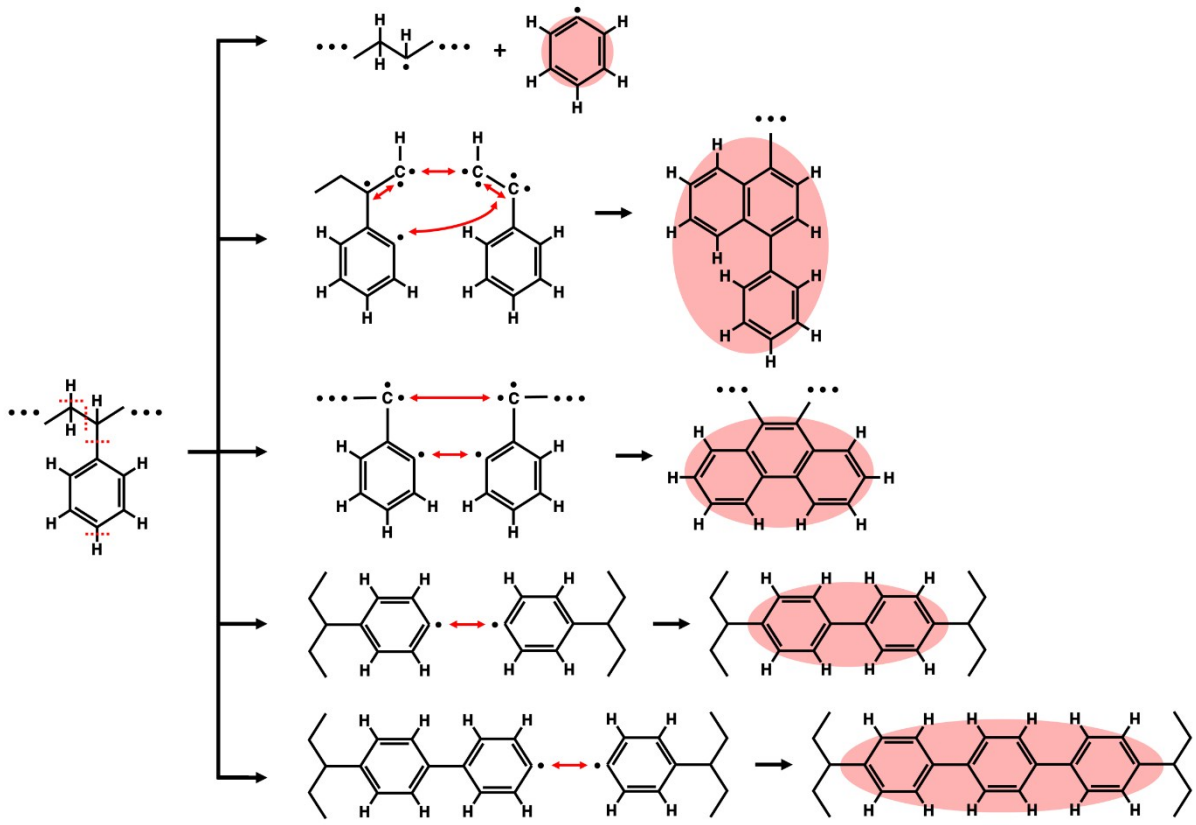

Fig. S6 Schematic illustration of the possible pathways to formation of polycyclic structures by electron irradiation of PS.

Materials	SPF	PA
Avobenzone (3 %)	2.7	3.1
Oxybenzone (6 %)	1.5	1.5
Octocrylene (10 %)	7.2	3.3
Octinoxate (7.5 %)	8.1	1.9
Octisalate (5 %)	1.9	1.1
Homosalate (15 %)	2.3	1.2
TiO2 (25 %)	3.5	3.3
ZnO (25 %)	3.2	3.3

Table S1 The *in vitro* SPF and PA values of test samples containing most commonly used sunscreen active ingredients at US FDA-approved maximum concentrations. Since commercial sunscreens consist of multiple different UV-absorbing ingredients, the corresponding SPF (PA) value of each ingredient was determined at its maximum allowed concentration employing the same previously used sunscreen base for comparison with the SPF (PA) values of electron-irradiated polymer NPs.