

Supporting Information

Cross-Linked Fluorescent Supramolecular Nanoparticles as Finite Tattoo Pigments with Controllable Intradermal Retention Times

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1. Preparation of FSNPs with various sizes by changing the Ad-PAMAM/CD-PEI ratio (w/w)

The size of FSNPs was controlled by altering the Ad-PAMAM/CD-PEI ratio (w/w).¹ To a solution of Ad-PEG (1.836 mg/mL) in 485- μ L of PBS buffer, CD-PEI (0.8 mg/mL) was injected under vigorous stirring. MPS-PPV (0.005 – 0.15 mg/mL) was then added sequentially, and the mixture solution was stirred vigorously for 2 min. A 5- μ L aliquot of DMSO containing Ad-PAMAM (0.15 – 1.6 mg/mL) was added into the mixture solution to obtain FSNPs. Addition of Ad-PAMAM over 1.6 mg/mL (*i.e.*, ratio of Ad-PAMAM/CD-PEI = 2.0:1) resulted in the aggregation of FSNPs. The hydrodynamic size of FSNPs was measured using dynamic light scattering (DLS; Figure S1a). The morphology and size of FSNPs were examined using transmission electron microscopy (TEM; Figure S1b).

The hydrodynamic size of FSNPs increased from 98 ± 9 nm to 110 ± 11 , 128 ± 13 , 171 ± 6 , 240 ± 14 , 410 ± 39 , and 670 ± 43 nm as the ratio of Ad-PAMAM/CD-PEI changed from 0.19 to 0.25, 0.5, 1.0, 1.5, 1.7, and 2.0, respectively. The sizes of FSNPs observed by TEM were 65 ± 5 , 90 ± 8 , 110 ± 12 , 160 ± 14 , 290 ± 20 and 498 ± 37 nm for FSNPs with hydrodynamic sizes of 98, 110, 128, 171, 240, 410, and 670 nm, respectively. The reduced sizes obtained by TEM were attributed to the dehydration of FSNPs during TEM sample preparation.

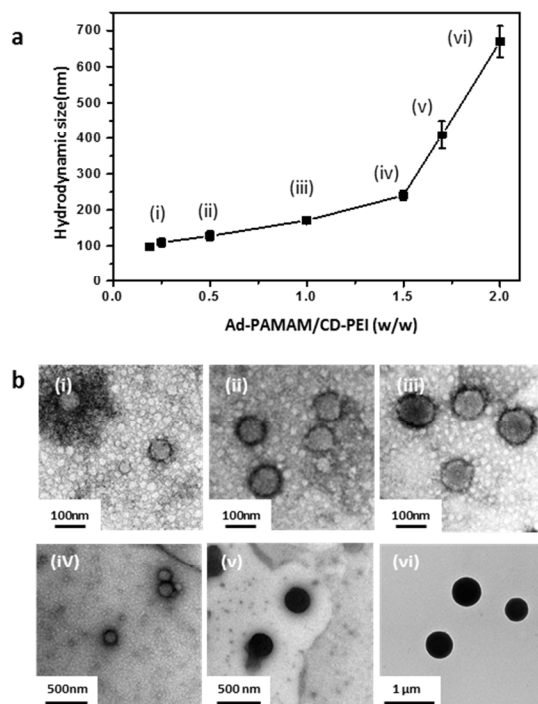


Figure S1. Hydrodynamic size (a) and TEM images (b) of FSNPs with various Ad-PAMAM/CD-PEI ratios (w/w). TEM images of FSNPs with Ad-PAMAM/CD-PEI ratios (w/w) of (i) 0.19, (ii) 0.5, (iii) 1.0, (iv) 1.5, (v) 1.7, and (vi) 2.

2. Absorption spectra of FSNPs with various Ad-PAMAM/CD-PEI ratios (w/w)

The optical properties of FSNPs with various Ad-PAMAM/CD-PEI ratio (w/w) were examined. The UV-Vis absorption of free MPS-PPV and FSNPs was obtained with a Hitachi UV-Vis system (Hitachi U-4100 spectrophotometer, Hitachi, Japan). The absorption spectra was obtained from 350 nm to 800 nm with a scan speed of 10 nm /sec with 1 nm intervals.

As shown in Figure S2, FSNPs show a higher absorption compared to free MPS-PPV. In addition, FSNPs with a high content of Ad-PAMAM show higher absorption.²⁻³

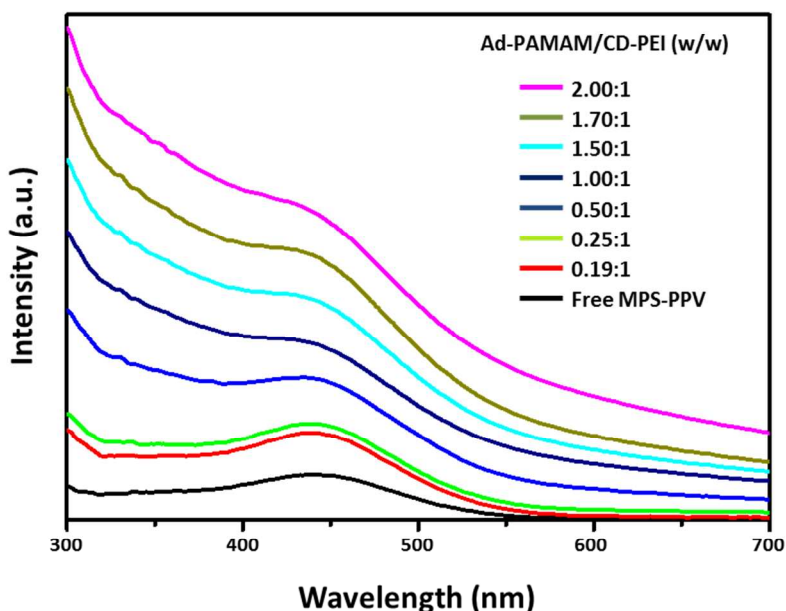


Figure S2. Absorption spectra of FSNPs with various ratio of Ad-PAMAM and CD-PEI (w/w)

3. Tattooing with commercially available tattoo dyes

Six- to eight-weeks-old female BALB/c nude mice were purchased from the Envigo (Livermore, CA, USA). All animal manipulations were performed with sterile technique and approved by the Institutional Animal Care and Use Committee of University of Southern California. Commercially available tattoo pigments, *i.e.*, ZnO (SkinCandy Tattoo Supply, San Francisco, CA, USA; 200 mg/mL), fluorescent dye captured polymethylmethacrylate (PMMA) particles (Chameleon Tattoo & Body Piercing, Cambridge, MA, USA; 500 mg/mL) and India ink (Speedball Art Products, NC, USA; 200 mg/mL), were utilized as control groups (Figure S3 a-c). After the mice were anesthetized with 2% isoflurane in a heated (37 °C) induction chamber, commercially available tattoo dye solutions were dropped on the mouse skin (nu/nu). The mouse skin with dye solution was poked with a 25 G needle to deposit the FSNPs into the dermis. The strong fluorescent signal of the tattooed ZnO and PMMA was observed under a UV light irradiation (365 nm; Figure S3d). The black spot from tattooed India ink is clearly visible after tattooing (Figure S3d). Signals from all of commercially available tattoo pigments could be observed for 108 days (15 weeks and 3 days).

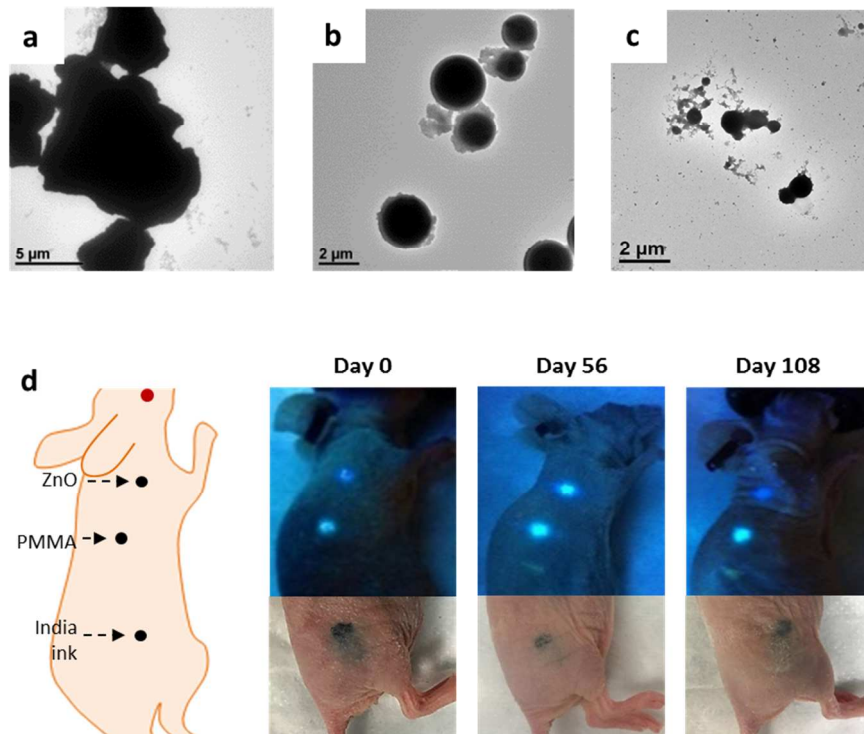


Figure S3. TEM images of (a) ZnO, (b) PMMA, and (c) India ink. (d) Photographs of ZnO, PMMA, and India ink tattooed mouse under UV light irradiation (ZnO and PMMA) and under ambient light (India ink)

4. Pathological study of commercially available tattoo dyes

Pathological analysis was carried out at the second day after dye treatments. Untreated skin tissues of the mice were used as control groups. Skin tissues were fixed with formalin and blocked with paraffin before the sectioning. The slices of skin tissues were stained with hematoxylin and eosin (H&E) and examined on an Aperio ScanScope AT microscope (Leica biosystem, USA). In H&E images (Figure S4), a large population of inflammation cells were observed in the skin tissues with ZnO and PMMA treatment on day two after tattooing.

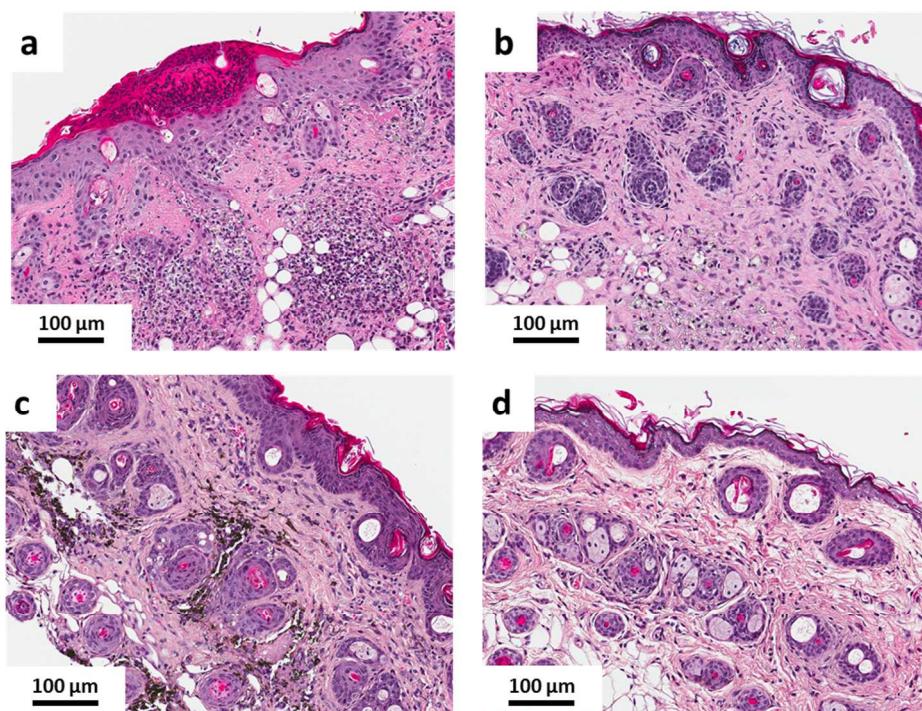


Figure S4. Pathological images (H&E staining) of skin treated with (a) ZnO, (b) PMMA, (c) and

India ink on day two after dye treatments (magnification: 100×). (d) Non-treated skin was utilized as control.

References

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