PAPER

Hyaluronate modified upconversion nanoparticles for near infrared light-triggered on–off tattoo systems†

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An *in vivo* on–off tattoo system was developed using upconversion nanoparticles conjugated with hyaluronate (HA-UCNPs). Two-photon microscopy clearly visualized the transdermal delivery of HA-UCNPs into the deep skin tissue. Upon near-infrared light irradiation, invisible HA-UCNPs in the skin were visualized showing the feasibility as a new on–off tattoo system.

Upconversion nanoparticles (UCNPs) doped with lanthanide ions are well-known as promising light delivery materials due to their ability to convert near-infrared (NIR) light to visible light. We can make UCNP to emit dynamically fine-tuned full colour light by adjusting the composition and the pulse-type of NIR laser. NIR light has a relatively high penetration depth compared with visible light because most biomolecules have low absorbance in the NIR window (700–1100 nm).[1] The long wavelength NIR light is also biocompatible for the tissue due to their low energy.[2] Along with the remarkable optical excitation and emission properties, and the absence of auto-fluorescence in biological tissues under NIR light excitation,[3,4] UCNP systems have emerged as a light delivery nanoplatform for photodynamic therapy, photothermal therapy and various photomedicines. It is a crucial issue to achieve non-invasive and controllable light delivery into the deep tissue.

In order for transdermal delivery, UCNP was conjugated with hyaluronate (HA) which has been widely used for a variety of biomedical applications. Recently, HA has been exploited as an effective transdermal delivery carrier of nanomaterials and biopharmaceuticals.[5] HA is hygroscopic, but has a hydrophobic patch domain on the molecular backbone.[6] Consequently, HA facilitates hydrating stratum corneum (SC) composed of the lipid bilayer and enhancing the penetration of tethered payloads through the skin. This outstanding feature of HA might enable the effective transdermal delivery of human growth hormone (hGH)[7] and nanomaterials like nano graphene oxide[8] and gold (Au) nanoparticles.[9] After topical delivery, two-photon microscopy clearly visualized the

penetration of FITC-labelled HA-hGH conjugate into skin tissues of mice. Furthermore, Au nanorods could be transdermally delivered for theranostic applications.[9] From the results, HA appeared to be used as a feasible transdermal delivery carrier of nanomaterials with greatly improved compliance.

Here, we developed HA modified UCNP for biocompatible transdermal light delivery. UCNP of NaYF$_4$:Yb/Er (Y : Yb : Er = 69 : 28 : 3) was synthesized by hydrothermal decomposition method as reported elsewhere.[10] HA-UCNP could be used as a new kind of on–off tattoo system which can be visualized in a controlled manner by irradiating NIR laser. Scheme 1a shows the simple steps of on–off tattoo system. First, HA-UCNP was synthesized and delivered on the dorsal skin of balb/c nude mice in a desired shape. After transdermal delivery for 40 min, the area loaded with HA-UCNP was cleaned by washing and visualized by the fluorescence of specific shapes after irradiating 980 nm laser. As shown in Scheme 1b, UCNP was surface-modified with poly(allylamine) (PAAm) and then conjugated with HA using the EDC/sulfo-NHS chemistry. Oleic acids on the surface of UCNP were replaced with PAAm by the ligand exchange to prepare UCNP-PAAm with amine groups.[11]
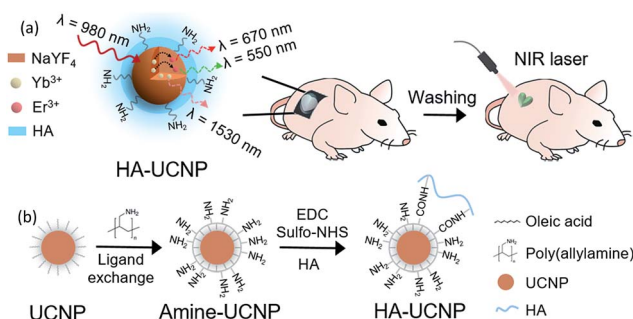
Scheme 1 (a) Schematic illustration for on–off tattoo system using HA-UCNP and NIR laser, and (b) synthetic chemistry of HA-UCNP.

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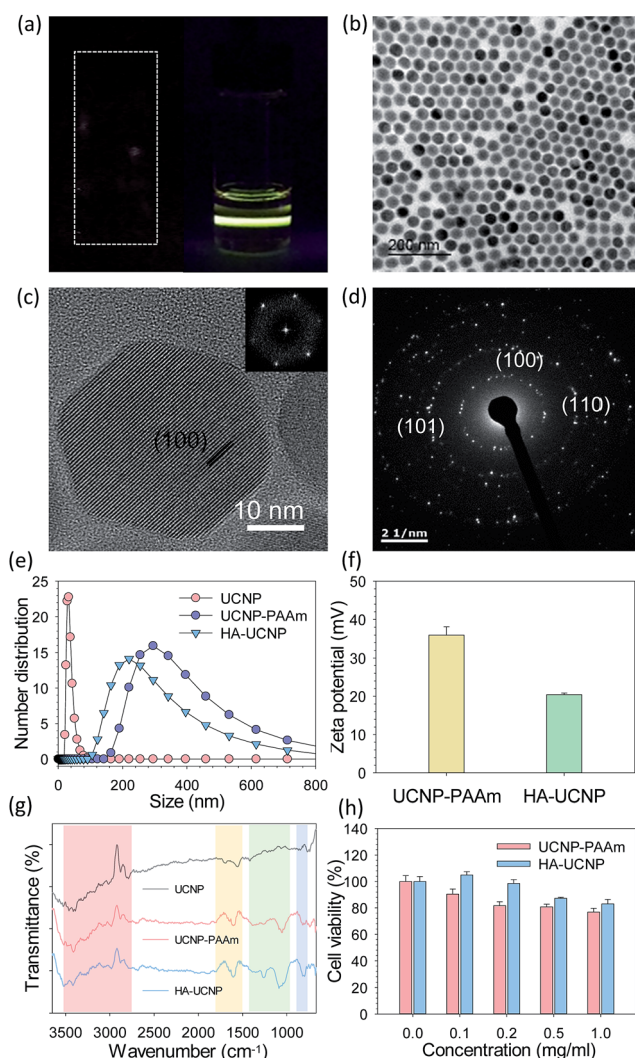
adjusted by changing their compositions[17–19] or temperature[20,21] in the synthetic steps. However, UCNP has the advantage to be colour-controlled by the excitation laser intensity due to several kinds of lanthanide ions which have multiple energy levels. This optical characteristics of UCNP is suitable to construct diverse colour fluorescence emitting on–off tattoo systems. After *in vitro* characterization, biocompatible HA-UCNP was assessed as an on–off tattoo system in 6 weeks old balb/c nude mice. HA-UCNP was topically administered onto the heart-shaped frame on the skin of nude mice for 40 min. After complete washing, the fluorescence was visualized by 980 nm NIR laser irradiation which was collimated by the expanded lens (Fig. 3). Upon irradiating NIR light, the invisible heart pattern containing HA-UCNP in the skin appeared as a new type of on–off tattoo
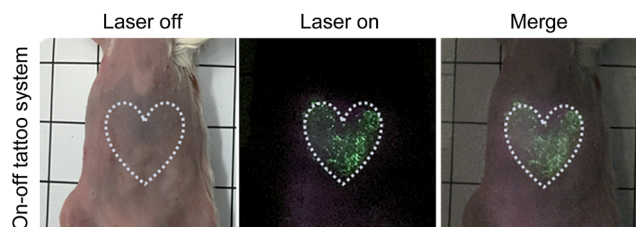

Fig. 3 *In vivo* on–off tattoo system which can be visualized in a controlled manner by irradiating 980 nm laser after transdermal delivery of HA-UCNP ($n = 3$).
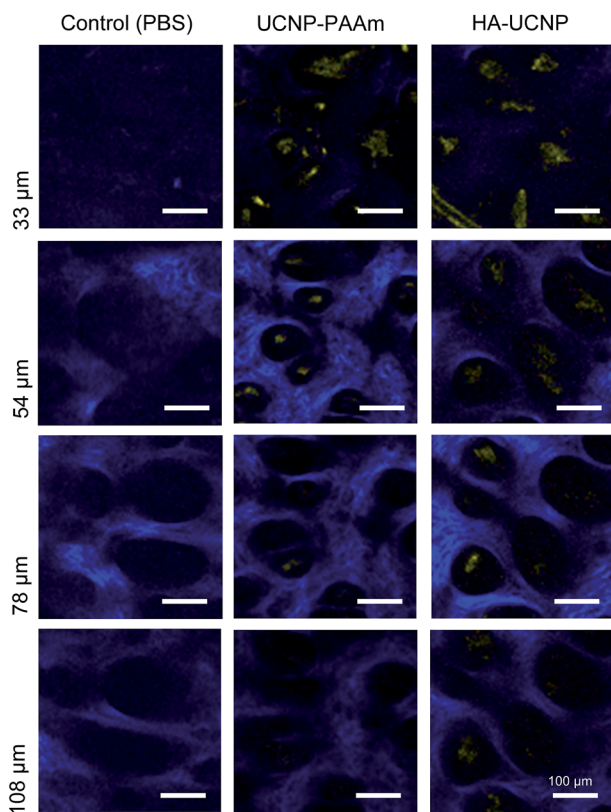

Fig. 4 Two-photon microscopic images of PBS, UCNP-PAAm and HA-UCNP transdermally delivered into the collagen layer of nude mice.

system using non-invasive transdermal delivery and simple NIR light irradiation. The on–off tattoo system was also demonstrated in a triangle shape (Fig. S3, ESI†). Animals were maintained in accordance with the guidelines of the Korea Food and Drug Administration, and all procedures with animals were approved by the Institutional Animal Care and Use Committee (IACUC) at the POSTECH Biotech Center.

Finally, two-photon microscopy was performed to assess the transdermal delivery of HA-UCNP. Fig. 4 shows two-photon microscopic images of PBS as a control, UCNP-PAAm and HA-UCNP topically delivered into the collagen layer of nude mice with 1050 nm laser excitation. The blue fluorescence represents collagen layers in the dermis by the second harmonic generation.[9] Compared with UCNP-PAAm, a significant amount of HA-UCNPs were observed over the depth of *ca.* 100 μm in the dorsal skin tissue. The transdermal delivery of UCNP-PAAm and HA-UCNP with increasing penetration depth was quantitatively compared by the two-photon microscopy (Fig. S4, ESI†). The penetration spectra of HA-UCNP was shifted into the deeper depth than that of UCNP-PAAm. While positively charged UCNP-PAAm might be transdermally delivered by the nano size effect, HA-UCNP might be more effectively delivered than UCNP-PAAm through the skin by the facilitation of HA with a hydrophobic patch domain. In other words, HA can enhance the biocompatibility and the penetration of UCNP. Recently, UCNP has been widely investigated for multi-modal bioimaging[22–24] and theranostic applications.[25–27] Taken together, this platform technology might be successfully exploited for various biomedical applications.

Conclusions

HA-UCNP was successfully developed as a multi-colour on–off tattoo system. The conjugation of UCNP with HA was confirmed by DLS, FT-IR and HiLyte amine dye labelling. MTT assay revealed the improved biocompatibility of UCNP-PAAm after conjugation with HA. According to two-photon microscopy, HA-UCNP appeared to penetrate into the collagen layer of the dermis in nude mice. The fluorescence of invisible HA-UCNP patterned in a specific shape could be visualized by simple NIR laser irradiation. This new platform technology for on–off tattoo systems might be further applied to various photomedicines.

Acknowledgements

Notes and references

1 J. V. Frangioni, *Curr. Opin. Chem. Biol.*, 2003, **7**, 626–634.
2 A. M. Smith, M. C. Mancini and S. Nie, *Nat. Nanotechnol.*, 2009, **4**, 710–711.

3 S. Wang, L. Zhang, C. Dong, L. Su, H. Wang and J. Chang, *Chem. Commun.*, 2015, **51**, 406.

4 G. Chen, H. Qiu, P. N. Prasad and X. Chen, *Chem. Rev.*, 2014, **114**, 5161–5214.

5 H. S. Jung, K. S. Kim, S. H. Yun and S. K. Hahn, *Nanomedicine*, 2014, **9**, 743–745.

6 I. C. M. Dea, R. Moorhouse, D. A. Rees, S. Arnott, J. M. Guss and E. A. Balazs, *Science*, 1973, **179**, 560–562.

7 J. A. Yang, E. S. Kim, J. H. Kwon, H. Kim, J. H. Shin, S. H. Yun, *et al.*, *Biomaterials*, 2012, **33**, 5947–5954.

8 H. S. Jung, W. H. Kong, D. K. Sung, M. Y. Lee, S. E. Beack, D. H. Keum, K. S. Kim, S. H. Yun and S. K. Hahn, *ACS Nano*, 2014, **8**, 260–268.

9 H. Lee, J. H. Lee, J. Kim, J. H. Mun, J. Chung, H. Koo, C. Kim, S. H. Yun and S. K Hahn, *ACS Appl. Mater. Interfaces*, 2016, **8**, 32202–32210.

10 M. K. Gnanasammandhan, N. M. Idris, A. Kansal, K. Huang and Y. Zhang, *Nat. Protoc.*, 2016, **11**, 688–713.

11 L. Xia, X. Kong, X. Liu, L. Tu, Y. Zhang, Y. Chang, K. Liu, D. Shen, H. Zhao and H. Zhang, *Biomaterials*, 2014, **35**, 4146–4156.

12 A. D. Ostrowski, E. M. Chan, D. J. Gargas, E. N. Katz, G. Han, P. J. Schuck, D. J. Milliron and B. E. Cohen, *ACS Nano*, 2012, **6**, 2686–2692.

13 C. D. Laboda and C. L. Dwyer, *Adv. Funct. Mater.*, 2016, **26**, 2866–2874.

14 H. Lee, M. Y. Lee, S. H. Bhang, B. S. Kim, Y. S. Kim, J. H. Ju, K. S. Kim and S. K. Hahn, *ACS Nano*, 2014, **8**, 4790–4798.

15 B. Zhou, B. Shi, D. Jin and X. Liu, *Nat. Nanotechnol.*, 2015, **10**, 924–936.

16 F. Wang, D. Banerjee, Y. Liu, X. Chen and X. Liu, *Analyst*, 2010, **135**, 1797–2160.

17 Y. Lu, J. Zhao, R. Zhang, Y. Liu, D. Liu, E. M. Goldys, X. Yang, P. Xi, A. Sunna, J. Lu, Y. Shi, R. C. Leif, Y. Huo, J. Shen, J. A. Piper, J. P. Robinson and D. Jin, *Nat. Photonics*, 2014, **8**, 32–36.

18 G. Tian, Z. Hu, L. Zhou, W. Yin, X. Liu, L. Yan, S. Jin, W. Ren, G. Xing, S. Li and Y. Zhao, *Adv. Mater.*, 2012, **24**, 1226–1231.

19 F. Wang and X. Liu, *J. Am. Chem. Soc.*, 2008, **130**, 5642–5643.

20 W. Niu, S. Wu, S. Zhang and L. Li, *Chem. Commun.*, 2010, **46**, 3908–3910.

21 S. Zheng, G. Ren, C. Xu and Q. Yang, *CrystEngComm*, 2011, **13**, 1384–1390.

22 Z. Liu, F. Pu, S. Huang, Q. Yuan, J. Ren and X. Qu, *Biomaterials*, 2013, **34**, 1712–1721.

23 Z. Liu, E. Ju, J. Liu, Y. Du, Z. Li, Q. Yuan, J. Ren and X. Qu, *Biomaterials*, 2013, **34**, 7444–7452.

24 Z. Liu, K. Dong, J. Liu, X. Han, J. Ren and X. Qu, *Small*, 2014, **10**, 2429–2438.

25 M. Sun, L. Xu, W. Ma, X. Wu, H. Kuang, L. Wang and C. Xu, *Adv. Mater.*, 2015, **28**, 898–904.

26 X. Wu, L. Xu, W. Ma, L. Liu, H. Kuang, N. A. Kotov and C. Xu, *Adv. Mater.*, 2016, **28**, 5907–5915.

27 S. Li, L. Xu, W. Ma, X. Wu, M. Sun, H. Kuang, L. Wang, L. A. Kotov and C. Xu, *J. Am. Chem. Soc.*, 2016, **138**, 306–312.