Noninvasive Photochemical Epithelial Tissue Bonding Using Upconversion Nanoparticles / Hyaluronate – Rose Bengal Conjugate Complex

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Introduction

Photomedicine is a future technology using light for diverse biomedical applications to laser surgery, optical diagnosis and cancer therapy. Among these applications, photochemical tissue bonding (PTB) is a promising dye-assisted photochemical technique for wound closure using rose bengal (RB) dyes via collagen crosslinking in the damaged skin tissue. Upconversion nanoparticles (UCNPs) can be used as a facile light source to replace the conventional implantable light-guiding materials with the unique optical property converting tissue penetrating near-infrared (NIR) light to visible light. Here, we developed a versatile deep tissue photomedicine of poly(allylamine), PAAm modified upconversion nanoparticle / hyaluronate - rose bengal (UCNP/PAAm/HA-RB) conjugate complex for PTB after its transdermal delivery.

Methods

UCNP/PAAm/HA-RB conjugate complex was prepared by simple mixing of UCNP with PAAm and HA-RB conjugate via electrostatic interaction. The UCNP/PAAm/HA-RB conjugate complex was analyzed by high resolution - transmission electron microscopy (HR-TEM), the energy dispersive spectroscopy, dynamic light scattering and UV/vis spectrophotometry. The cytotoxicity of HA-RB conjugate, UCNP/PAAm, and the UCNP/PAAm/HA-RB conjugate complex was assessed in NIH3T3 cells using the MTT assay. For light propagation assessment, the porcine skin (2 cm × 1 cm) was prepared and illuminated by green and NIR light (*ca.* 500 mW/cm^2). Collagen fibrillogenesis test for the rate and extent of collagen crosslinking was carried out spectrophotometrically. For *in vivo* animal tissue bonding test, 50 μl samples were treated on the incision on balb/c dorsal skin and NIR light was illuminated for 20 min. *Ex vivo* tensile strength of porcine skin was measured by using a universal testing machine equipped with a 10 N load cell.

Results

The UCNP/PAAm/HA-RB conjugate complex was formed by the electrostatic interaction between positively charged UCNP/PAAm and negatively charged HA-RB conjugate. According to HR-TEM and X-ray diffraction spectroscopy, UCNPs appeared to have a (100) hexagonal crystal lattice with a uniform size of 30.43 ± 2.10 nm. UV/vis spectrophotometry and fluorometry showed the overlap between the absorbance wavelength of the HA-RB conjugate and the emission wavelength of UCNP/PAAm. The light propagation test showed that the green laser could not reach into the dermis layer, but was scattered on the stratum corneum (SC) layer. In contrast, the NIR laser penetrated into the dermal layer of skin tissue treated with the complex for 30 min and converted to green light by UCNPs in the complex. In the collagen fibrillogenesis test, the collagen fibrillogenesis rate increased significantly for the case of the UCNP/PAAm/HA-RB conjugate complex with 980 nm NIR light illumination. Finally, the PTB of UCNP/PAAm/HA-RB conjugate complex with NIR illumination was confirmed by *in vivo* experiments for 3 days and *ex vivo* tensile strength test after sacrifice. The UCNP/PAAm/HA-RB conjugate complex could be transdermally delivered into the deep and wide area of incision due to HA. Upon NIR light illumination, UCNP convert the NIR to green light for the activation of RB in the HA-RB, inducing the collagen crosslinking for accelerated sutureless tissue bonding. The UCNP/PAAm/HA-RB conjugate complex resulted in a higher tensile strength than that of the conventional suturing without allergic side effects.

Conclusions

We successfully demonstrated the feasibility of the UCNP/PAAm/HA-RB conjugate complex for noninvasive PTB in deep tissue under NIR light illumination. HA in the conjugate appeared to facilitate the penetration of RB into a deep and wide area from the boundary of incision. UCNPs in the complex showed the effective light-guiding ability into the deep tissue as an alternative to implantable or injectable light-guiding materials. Two-photon microscopy revealed the facilitated transdermal delivery of the complex into the dermal tissue. The activated RB in the HA-RB conjugate by the green light induced radical formation for the crosslinking of incised collagen matrix. After confirming *in vitro* collagen fibrillogenesis, we performed *ex vivo* and *in vivo* skin tissue bonding, which revealed the photochemically accelerated photochemical tissue bonding effect of the UCNP/PAAm/HA-RB conjugate complex under noninvasive NIR light illumination compared to the conventional invasive treatment via suturing and stapling. This platform technology of the UCNP/PAAm/HA-RB conjugate complex might be successfully applied for the development of various futuristic photomedicines.