A Bioinspired, Sustained-Release Material in Response to Internal Signals for Biphasic Chemical Sensing in Wound Therapy

Abstract

Biofluorescence in living entities is a functional process associated with information conveyance; whereas the capacity to respond to internal physiological signals is a unique property of a cell. By integrating these two biological features into materials design, a bioinspired material, namely CPS, is developed. Contrary to conventional luminescent polymeric systems whose emission comes from π-conjugated structures, this material displays clusterization-triggered emission. In the preclinical trial on a dermal punch model of tissue repair, it successfully increases the rate of wound closure, reduces inflammatory cell infiltration, and enhances collagen deposition. It can also relay changes in internal chemical signals into changes in its intrinsic luminescence for biphasic chemical sensing to prevent possible occurrence of skin hyperpigmentation caused by minocycline hydrochloride in wound therapy. Together with its ease of fabrication, high biocompatibility, high drug loading efficiency, and high release sustainability, CPS shows high potential to be developed into an intelligent solid-state device for wound treatment in the future.

1 Introduction

Biofluorescence is a process in which electromagnetic radiation at one wavelength is first absorbed by an organism, followed by the reemission of the radiation at a longer and lower energy wavelength, leading to the occurrence of green, orange, and red emission coloration in organisms. An earlier study has revealed that, because of the presence of yellow intraocular filters in lenses and corneas,[1] some fishes (including lizardfishes, sharks, scorpionfishes, and flatfishes) can visualize the visual contrast and patterns created by the biofluorescence of fishes of the same species, enabling biofluorescence to be used to disclose spatial information to other fishes while leaving those fishes unseen to predators that lack this visual specialization.[2] A similar situation occurs in frogs, whose biofluorescence conveys messages about location to amphibians of the same species under nocturnal conditions while enabling the frogs to remain camouflaged to predators.[3] These observations point to the role played by biofluorescence in information conveyance for intra- and interspecific communication. Meanwhile, cells are the basic unit of life. In response to signals (including the nutrient level and shear stress) associated with environmental changes,[4] they can dynamically alter their behavior biomechanically or biochemically.[5] Inspired by this unique signal processing property in cells as well as by the possible role played by biofluorescence in information conveyance,[6] here we develop a bioinspired intelligent material, namely CPS, which can relay changes in internal chemical signals into changes in luminescence externally to convey messages about the real-time status of the material (**Figure 1A**). This enables persistent tracking of the process of local drug delivery in wound therapy.

Over the years, although copious delivery-tracked systems have been reported in the literature,[7] materials that enable monitoring of multiple factors simultaneously during the course of the treatment are lacking. Our bioinspired material may fill this technical gap by

enabling comprehensive and biphasic drug monitoring during the full course of the treatment. In addition, at the moment most of the existing delivery-tracked systems rely on the use of complex equipment [e.g., positron emission tomography and single photon emission computed tomography] and tedious measurement procedures.[7] This hinders the use of these systems in routine clinical practice. This situation is compounded by the fact that many of those systems require the use of contrast enhancers or metallic nanocrystals for proper functioning.[8] This interferes with the loading and release of the therapeutic agent, and increases the toxicity of the drug-loaded system if any toxic contrast enhancer and nanocrystal is released along with the drug to the body.[9] Contrary to these systems, the status ofthe chemical entities (in this case drug molecules) loaded inside our bioinspired material can be tracked solely based on changes in the intrinsic luminescence of the material *per se*. Neither complex equipment nor additional contrast enhancers is required during the tracking process. Importantly, similar to a living cell which can respond to internal signals (e.g., changes in the concentration and phosphorylation status of signaling molecules inside), our bioinspired material also responds to internal chemical signals (in particular, changes in the quantity and oxidative status of the loaded drug molecules). This is different from existing luminescent systems and probes, which respond only to external signals provided by the environment.

Apart from these, contrary to conventional luminescent polymeric materials, whose emission comes from the π-conjugated structure, the luminescence of our bioinspired material is designed to be based solely on clusterization-triggered emission (CTE),[10] which is an aggregation-induced emission-like process thought to be caused by interactions among electron-rich heteroatoms.[11] The occurrence of CTE has been reported in oligo(maleic anhydride)s, in which intrachain noncovalent interactions of the carbonyl groups enable the interpenetration of the lone pair of one carbonyl group into the empty π^* orbital of another carbonyl group, resulting in the hybridization of the orbitals and ultimately the release of the excited energy as luminescent emission.[12, 13] A similar phenomenon of luminescence has also been shown in natural polymers (such as cellulose and sodium alginate),[14, 15] whose emission is expected to stem from through-space nonbonding interactions between heteroatoms, with such interactions narrowing down the energy gap between the highest occupied molecular orbitals and the lowest unoccupied molecular orbitals to render the polymers luminescent.[16] The incorporation of CTE into our materials design makes our bioinspired system more favorable than conventional luminescent organic materials for clinical use. First, our polymer is free of the π-conjugated structure. It does not contain the planar conformation (which are inherently linked to the conjugated electronic structure) of conventional luminescent polymers. For this, while other luminescent polymers experience quenching of the emission in the aggregated state and hence fail to serve as a solid-state device for drug loading and release,[17] our material can go beyond their limit by serving as a multifunctional drug carrier with strong luminescence emission. In addition, extended conjugation in conventional luminescent polymers is often accompanied with greater structural complexity and laborious synthetic procedures. On contrary, our bioinspired material can be generated simply by using a derivative of chitosan (CS), which is accessible at low cost and is shown to be highly biocompatible.[18] This makes our material convenient to be applied in the clinical setting.

2 Results and Discussion

The generation of CPS starts with the activation of the carboxyl groups of CS by 1,1'-carbodiimidazole (CDI). Activated CS is crosslinked by poly(ethylenimine) (PEI) via urethane linkages to form PS (Figure S1, Supporting Information). Finally, CPS is generated upon complexation of PS with sodium carboxymethyl cellulose (SC). This complexation process is ionic in nature. It governs the dramatic difference between our final material and the CS counterpart (designated as CCS) in both drug delivery performance and emissive properties. It provides CPS with the capacity of drug loading and sustained release, and also enables CPS to attain the unique emissive properties needed for its sensitivity to internal signals. Importantly, polyelectrolyte complexation involved in the generation of CPS can be done at the time and site of use, and is convenient for applications in the subsequent preclinical trial. The structures of PS and CPS are studied by using Fourier-transform infrared (FT-IR) spectroscopy. The spectrum of CS shows a broad peak at 2971–3700 cm^{-1} (Figure S2A, Supporting Information). This peak is assigned to O-H and N-H stretching vibrations. A peak is also found at 2890 cm^{-1}, due to C-H and C-N stretching vibrations. The peaks observed at 1590 and 1070 cm^{-1} are assigned to N-H bending vibrations and C-O-C stretching vibrations, respectively. After crosslinking by PEI, the intensity of the signal (1590 cm^{-1}) assigned to N-H bending vibrations increases. This indicates the increase in the amine content of CS after crosslinking by PEI. The success of crosslinking is further confirmed by the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of PS (Figure S2B, Supporting Information), which shows representative signals of PEI at around 2.5–2.7 ppm and also the signals of CS at 1.97 ppm ($NCOCH_3$), 3.13 ppm (H_2) and 3.59–3.84 ppm (H_3, H_4, H_5, H_6). In the spectrum of SC, a broad absorption band is found at 2955–3682 cm^{-1} due to O-H stretching vibrations. The band at 2918 cm^{-1} is assigned to C-H stretching vibrations. The signal at 1585 cm^{-1} demonstrates the presence of COO- groups. The bands at 1416 and 1005 cm^{-1} are attributed to -CH_2 scissoring vibrations and CHO-CH_2 stretching vibrations, respectively. These signals are also found in the spectrum of CPS.

Compared to CS, the solubility of PS is significantly higher (Figure S2C, Supporting Information). This is explained by the disruption of intra- and intermolecular hydrogen bonds in CS during covalent crosslinking by PEI, but is also contributed by the increase in the amount of amine groups, and hence the hydrophilicity of the resulting polymer, after the crosslinking process. As suggested by the ninhydrin test, there is an approximately fourfold increase in the mass percentage of nitrogen after the crosslinking process, changing from 8% in CS to around 30% in PS. Due to its higher amine content, PS shows higher pH buffering capacity as compared to CS (Figure S2D, Supporting Information). In the thermogravimetric analysis (TGA) curve of CS, a significant weight-loss stage is observed at 40–113 °C (Figure S2E, Supporting Information). It is attributed to the evaporation of physically absorbed or bound water. Another weight loss step occurs at the onset temperature of 256 °C, owing to the onset of chain degradation of CS. An extra weight-loss stage at 120–249 °C is found in the TGA curve of PS. This is caused by the decomposition of PEI. Compared to CS, the thermal stability of PS is lower, possibly because the presence of PEI changes the crystallinity of CS. After complexation with SC, the thermal stability increases due to an increase in the structural rigidity of the polymeric network.

The emissive properties of our bioinspired material stems largely from PS, whose absolute quantum yield in a 5% v/v acetic acid solution is estimated to be fourfold higher than that of the acetic acid solution of CS (Figure S3A,B, Supporting Information). Blue emission at 451 nm, with a photoluminescence (PL) excitation peak at 386 nm, is recorded for the CS solution (Figure S3C, Supporting Information). Compared to that of the CS solution, the PL peak of the PS solution displays a red shift from 451 to 480 nm. In addition, upon crosslinking by PEI, the average lifetime changes from 2.69 to 3.07 μs (Table S1 and Figure S3D, Supporting Information). The higher luminescence intensity of PS as compared to that of CS is attributed to the higher branching structure as well as the higher amine content of PS. The luminescence of PS, in which aromatic residues are absent, comes partly from the PEI moieties, which have been reported to enable the formation of amine rich nanoclusters and to increase the likelihood of the occurrence of electron–hole recombination processes in which correlated electron–hole exciton states between localized states of electrons and holes are involved.[19] The intrinsic luminescence of PS can also be attributed to through-space electronic communication mediated by short contacts, such as N···N, N···C O, and C O···C O,[10, 20] and to hydrogen bonds formed between functional groups such as C O and N H. For the latter, earlier studies revealed that hydrogel bonding is not only a form of electrostatic interactions but can also cause electron density delocalization,[21] resulting in inter-intramolecular through-space conjugation. Such conjugation leads to the formation of a narrow bandgap that serves as a source of clusteroluminescence.[10] The occurrence of CTE is confirmed in Figure **1B**,**C**, in which the luminescence intensity, as well as the average lifetime, of the PS solution is positively related to the polymer concentration. This is an important feature of CTE as reported by earlier studies.[13, 15, 22]

Upon complexation with SC, the luminescence of CS is almost completely quenched; whereas PS is still highly luminescent (Figure S3E,F, Supporting Information). Energy transfer from CS or PS to SC is unlikely, owing to energy disparity and the lack of overlap between the absorption of SC and the emission of CS or PS. The loss of luminescence from CS in CCS is attributed to the weak ionic interactions between CS and SC. The effect of CS dispersion (caused by an increase in the volume of the whole system upon the addition of SC), therefore, outweighs the effect of CS aggregation led by polyelectrolyte complexation, leading to the loss of luminescent properties in CCS. On contrary, PS has a substantially higher amine content and a highly branched structure. It can complex with SC effectively due to strong electrostatic interactions. CTE from PS is, therefore, enhanced, enabling CPS to be used as a bioinspired intelligent material as depicted in the later part of this study. Intense blue emission at 490 nm, with a PL excitation peak at 380 nm, is found from CPS (Figure S3G, Supporting Information). The occurrence of a redshift in the PL spectrum of PS after complexation with SC is partly attributed to electron transfer between PS and SC and also to molecular aggregation promoted by interchain backbone interactions.[23]

The apparent viscosity of CCS and that of CPS are higher at a low shear rate than at a high shear rate (Figure S4A, Supporting Information). This indicates that these materials exhibit pseudoplastic behavior. The viscosity of CPS is slightly higher than that of CCS. This is explained by the larger amount of amine groups available in PS for electrostatic interactions with the carboxyl groups of SC, leading to higher mechanical strength of the

material formed. Changes in G' and G'' values of CCS and CPS at different shear rates are shown in Figure S4B (Supporting Information). The G' values are found to be higher than the corresponding G'' values for all samples, indicating that both CCS and CPS display solid-like behavior. Compared to CCS whose compressive strength is 0.414 MPa, the compressive strength of CPS is 1.2-fold higher (Figure S4C, Supporting Information). In addition, CPS shows higher maximum tensile strength, as well as a higher value of the elongation at breakage, in comparison with CCS (Figure S4D, Supporting Information). This is attributed to the increase in the strength of polyelectrolyte complexation, thereby enabling CPS to display better load transfer and to resist the sliding of molecular chains. All these suggest that CPS is a biomaterial that shows much stronger mechanical properties than that of CCS.

As shown by scanning electron microscopy (SEM), pristine CS shows a granular morphology (Figure S4E, Supporting Information). After covalent crosslinking, the granular morphology of CS is distorted, and is transited to a fibrillar morphology. This distortion is caused by an increase in the extent of molecular entanglement, which makes the morphology of the polymer fibrillar.[24] Compared to CCS, CPS displays a more compact structure. This is explained by the higher amine content of PS, which enables stronger interactions with SC in the matrix. Due to its more compact structure, diffusion of water molecules into the matrix of CPS is more difficult. This explains the lower water content, the lower swelling ratio, and the lower erosion rate of CPS as compared to CCS (Figure S4F–H, Supporting Information).

To apply to wound therapy, minocycline hydrochloride (MH) is physically trapped in the matrix of CPS during material fabrication to generate CPS/M (Figure **1D**). The cytotoxicity of CPS is examined in 3T3 mouse fibroblasts, HDF cells, and HaCaT cells. No significant loss of cell viability, and hence no acute cytotoxicity, is observed after 5 h of treatment with CCS or CPS (Figure **1E**). To determine potential chronic cytotoxicity, the viability of the treated cells is further studied after 24 h post-treatment incubation. No detectable cytotoxicity is observed in all concentrations tested. This illustrates the high safety profile of CPS in biomedical use. The high biocompatibility of CPS is demonstrated by its negligible hemolytic activity, too (Figure **1F**). Previous studies suggested that CS promotes hemolysis.[25] After complexation with SC, the hemolytic activity of CCS and CPS is much lower than that of CS and PS. This is partly because during the fabrication of CCS and CPS, electrostatic interactions with SC occur. Possible electrostatic interactions of CS and PS with the erythrocyte plasma membrane are, therefore, reduced. Apart from its high biocompatibility, the percentage of blood absorption mediated by CPS is around 20% higher than that of medical gauze (Figure **1G**). This ability may facilitate the maintenance of moisture balance during wound treatment,[26] and is favorable for the development of blood absorbent products for hemostatic applications.

Although there is no statistically significant difference between CPS and CCS in terms of their loading efficiency (LE) and encapsulation efficiency (EE), CPS displays higher release sustainability than CCS (Figure **1H**–**I**; and Figure S5, Supporting Information). This is attributed to the more compact structure of CPS, leading to a reduction in the swelling ratio, the water content, and the erosion rate. This may reduce the loss of the adsorbed drug molecules during drug loading and release. To examine the effect of changes

in the concentration of SC on the LE and EE, CPS with a higher or lower concentration of SC (designated as CPS-H and CPS-L, respectively) is produced. Because of the high molecular weight of SC as well as its rich contents of carboxyl and hydroxyl groups, the overall hydrophilicity of CCS and CPS is increased with the amount of SC added. This leads to a decrease in the release sustainability of the material, though the effect on the EE and LE is insignificant. To confirm the retention of the therapeutic effect of the drug after the drug loading process, both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria are used to examine the capacity of MH-loaded CCS (CCS/M) and MH-loaded CPS (CPS/M) in inhibiting the growth of bacteria. All samples (including CPS/M, CCS/M, and the control) lead to the formation of a zone of inhibition (Figure **1J**–**K**). This indicates that the impact of the drug loading process has little influence on the activity of MH.

The luminescence intensity of our material can respond to internal signals as what a living cell does. One of the signals that can lead to a change in the luminescence intensity is the change in the amount of MH molecules present inside our material (**Figure** 2A). The luminescence change is mediated largely by the capacity of the drug molecules inside to quench the luminescence of PS (Figure 2B). Due to the lack of overlap between MH absorption and CPS emission at 490 nm (Figure 2C), Förster resonance energy transfer between MH and CPS is not expected. In fact, as far as quenching is concerned, it can be either dynamic or static in nature. Dynamic quenching is governed by the linear Stern–Volmer equation.

On the other hand, static quenching is caused by the complexation of the quencher with the fluorophores in the ground state before excitation. The number of fluorophores is, therefore, reduced when the concentration of the quencher is increased. Static quenching, however, does not cause a reduction in the lifetime of the sample because uncomplexed fluorophores can still emit after excitation and hence show normal excited state properties. Under the situation of static quenching, a positive deviation from linearity is expected to occur in the Stern–Volmer plot. As shown by the Stern–Volmer plot of PS quenched by MH, the quenching mechanism is suggested to be static in nature (Figure **2D**).

Apart from the aforementioned, the luminescence intensity of our material can respond to changes in the oxidative status of MH molecules present inside, with the level of oxidation and the luminescence intensity being in a negative relationship (Figure **2E**). This is achieved by taking advantage of the fact that oxidized MH molecules inside our material cannot only quench the luminescence of PS directly via static quenching as the nonoxidized drug molecules do (Figure 2D), but they can also complex with SC, with the generated complex being able to impose additional quenching to the luminescence of PS in our bioinspired system (Figure **2F**–**G**). The occurrence of complexation between SC and oxidized MH is partially evidenced by changes in the ultraviolet–visible (UV–vis) absorption spectrum of SC after incubation with the oxidized drug (Figure **2H**). Owing to the higher overall quenching efficiency of oxidized MH, changes in the oxidative status of drug molecules inside our material can serve as the second internal signal that leads to changes in the luminescence of our material during its use in the subsequent preclinical trial.

To demonstrate the biomedical use of our material, a dermal punch model of tissue repair is adopted. An ideal material for applications in wound treatment is the one that can shorten the regeneration time, reduce the chance of infection, and ameliorate the pain experienced by patients. In this study, MH, which is a semisynthetic tetracycline possessing antibiotic properties against both gram-positive and gram-negative bacteria and has been examined clinically for the treatment of skin ulcers,[27] is loaded into CCS and CPS. The final concentration of the loaded drug in CCS and CPS is controlled to be 0.25% w/v, which is higher than the reported effective concentration (<0.0003%) of MH for antibacterial action *in vitro*.[28] This concentration of MH has been previously adopted by Choi and co-workers,[29] who loaded MH into a polyvinyl alcohol/CS hydrogel to treat wounds in mice and showed that the concentration of the drug is sufficient for wound treatment. No infection or necrosis in any of the treatment groups is found throughout the period of our study. On day 9, the wound in the CPS/M group is almost completely healed, while other groups are still in the recovery phase (Figure 3A; and Figure S6, Supporting Information). It is proposed that CPS facilitates wound healing partly because it maintains the release of MH in a stable and sustained manner, thereby enhancing the antibacterial effect and shortening the inflammatory stage to induce an earlier onset of the proliferative phase.

While the sustained release of MH is desired, MH can be oxidized easily in vivo to cause skin hyperpigmentation.[30] Three types of MH-caused hyperpigmentation have been identified clinically. Type I and II hyperpigmentation lead to the occurrence of localized blue-black discoloration over inflammatory regions and extremities, respectively; whereas type III hyperpigmentation leads to diffuse, muddy-brown discoloration of the sun-exposed area.[30] Such hyperpigmentation negatively impacts the outcome of wound treatment. Taken this into account, proper monitoring of the oxidative status of the drug is required, particularly after a drug-loaded dressing material has stayed in a wound for an extended period. This need is fulfilled by using our material, which displays a biphasic change in luminescence based on changes in signals inside (Figure **3B**–**D**). In the beginning of the treatment, MH molecules diffuse out of CPS, with the amount of MH molecules present inside decreasing continuously. This internal signal is sensed by CPS, leading to a gradual increase in the luminescence intensity. By examining the rate of luminescence recovery, the profile of sustained drug release can be tracked. After the recovery of the luminescence in the first phase, the recovered luminescence can serve as a probe in the second phase to enable the monitoring of another internal signal (i.e., changes in the degree of oxidation experienced by the loaded drug). By monitoring the decrease in the luminescence intensity, the oxidative status of loaded MH can be tracked. This enables skin hyperpigmentation potentially caused by treatment with MH to be prevented.

To confirm that changes in the luminescent intensity of CPS/M are predominately due to changes in the amount and oxidative status of MH present inside our material, we monitor changes in the luminescence intensity of CPS alone in the simulated body fluid (Figure S7, Supporting Information). The stable luminescence of CPS suggests that changes in the luminescence intensity observed during biphasic chemical sensing in wound therapy is only due to changes in internal signals (led by variations in the amount and oxidative status of the loaded drug) rather than being caused by components in a biological environment. In addition, to evaluate the quality of healing mediated by CPS as a carrier, histomorphologic changes in wounds are examined by hematoxylin and eosin (H&E)

staining and Masson's trichrome staining performed on tissue sections (Figure 3E). Compared to other groups, the wound in the CPS/M group shows less inflammatory cell infiltration, more collagen deposition, and more microvessel formation (Figure S8, Supporting Information). The lower degree of inflammatory cell infiltration indicates that inflammation is less severe in the wound treated with CPS/M. The higher level of collagen deposition and microvessel formation also suggest that, upon treatment with CPS/M, the healing of the wound is promoted.

3 Conclusion

Biofluorescence in living entities is a functional process for information conveyance; whereas the ability to respond to internal physiological signals is a feature of a living cell. This study integrates these two processes to generate CPS. In our preclinical trial, CPS effectively facilitates the action of the drug to enhance wound closure, and also successfully relays changes in internal signals (in particular, the amount and oxidative status of the loaded drug) to external phenotypes (in this case, variations in the luminescence intensity) so as to enable biphasic chemical sensing during wound treatment. Along with its good biocompatibility, high drug release sustainability and ease of preparation, CPS warrants further development as an intelligent and user-friendly system for use in drug delivery in the clinical context.

Acknowledgements

All animal experiments and ethical procedures were approved by the Ethical Committee of the Hong Kong Polytechnic University (accreditation number 180101). This work was supported by the University Research Facility for Chemical and Environmental Analysis (UCEA), Area of Excellent Grants (1-ZVGG) of the Hong Kong Polytechnic University, Shenzhen Science and Technology Innovation Committee (No. JCYJ20170818102436104), Natural Science Foundation of Guangdong Province (No. 2018A030310485), Chinese University of Hong Kong (Shenzhen) (No. PF01001421), and Research Grants Council of the Government of Hong Kong Special Administrative Region (No. C5012-15E).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] P. H. Heinermann,Exp. Biol.1984,43, 127.

[2] J. S. Sparks, R. C. Schelly, W. L. Smith, M. P. Davis, D. Tchernov, V. A.Pieribone, D. F. Gruber,PLoS One2014,9, e83259.

[3] C. Taboada, A. E. Brunetti, F. N. Pedron, F. Carnevale Neto, D. A. Es-trin, S. E. Bari, L. B. Chemes, N. Peporine Lopes, M. G. Lagorio, J.Faivovich,Proc. Natl. Acad. Sci. USA2017,114, 3672.

[4] a)D.H.Kim,D.D.Sarbassov,S.M.Ali,J.E.King,R.R.Latek,H.Erdjument-Bromage, P. Tempst, D. M. Sabatini,Cell2002,110, 163;b) J. Deng, K. H. Schoenbach, E. S. Buescher, P. S. Hair, P. M. Fox, S. J.Beebe,Biophys. J.2003,84, 2709; c) E. Tzima, M. Irani-Tehrani, W. B.Kiosses, E. Dejana, D. A. Schultz, B. Engelhardt, G. Cao, H. DeLisser,M. A. Schwartz,Nature2005,437, 426.

[5] a) S. Y. Tee, A. R. Bausch, P. A. Janmey,Curr. Biol.2009,19, R745; b) L.Lopez-Maury, S. Marguerat, J. Bahler,Nat. Rev. Genet.2008,9, 583.

[6] E. Reyssat, L. Mahadevan,J. R. Soc. Interface2009,6, 951.

[7] a) A. Ruiz-de-Angulo, A. Zabaleta, V. Gomez-Vallejo, J. Llop, J. C.Mareque-Rivas,ACS Nano2016,10, 1602; b) D. C. Wu, M. X. Wan,J.Controlled Release2015,213, E24.

[8] a) F. Z. Wu, H. X. Xu, Z. H. Zhu, X. Li, Y. H. Lu, T. Ma, X. J. Cai, R. Li,X. B. Wang, P. H. Xu,J. Wuhan Univ. Technol.2017,32, 1493; b) M. L.Chen, Y. J. He, X. W. Chen, J. H. Wang,Bioconjugate Chem.2013,24,387.

[9] a) J. H. Li, Y. Zhang, Q. Xiao, F. F. Tian, X. R. Liu, R. Li, G. Y. Zhao,F. L. Jiang, Y. Liu,J. Hazard. Mater.2011,194, 440; b) A. Hoshino, S.Hanada, K. Yamamoto,Arch. Toxicol.2011,85, 707; c) T. S. Hauck, R.E. Anderson, H. C. Fischer, S. Newbigging, W. C. W. Chan,Small2010,6, 138; d) M. Bottrill, M. Green,Chem. Commun.2011,47, 7039.

[10] H. Zhang, Z. Zhao, P. R. McGonigal, R. Ye, S. Liu, J. W. Y. Lam, R. T. K.Kwok,W.Z.Yuan,J.Xie,A.L.Rogach,B.Z.Tang,Mater. Today2020,32, 275.

[11] L. Viglianti, N. L. C. Leung, N. Xie, X. G. Gu, H. H. Y. Sung, Q. Miao,I. D. Williams, E. Licandro, B. Z. Tang,Chem. Sci.2017,8, 2629.

[12] a) K. M. Lee, W. Y. Cheng, C. Y. Chen, J. J. Shyue, C. C. Nieh, C. F.Chou, J. R. Lee, Y. Y. Lee, C. Y. Cheng, S. Y. Chang, T. C. Yang, M. C.Cheng, B. Y. Lin,Nat. Commun.2013,4, 1544; b) R. W. Newberry, R.T. Raines,ACS Chem. Biol.2014,9, 880.

[13] X.B.Zhou,W.W.Luo,H.Nie,L.G.Xu,R.R.Hu,Z.J.Zhao,A.J.Qin,B. Z. Tang,J. Mater. Chem. C2017,5, 4775.

[14] a) A. Khalid, L. Zhang, J.-P. Tetienne, A. N. Abraham, A. Poddar,R. Shukla, W. Shen, S. Tomljenovic-Hanic,APL Photonics2019,4,020803; b) W. F. Lai,J. Drug Delivery Sci. Technol.2020,59, 101916.

[15] X. Dou, Q. Zhou, X. Chen, Y. Tan, X. He, P. Lu, K. Sui, B. Z. Tang, Y.Zhang, W. Z. Yuan,Biomacromolecules2018,19, 2014.

[16] a) R. Hoffmann,Acc. Chem. Res.1971,4,1;b)X.G.Guo,J.Quinn,Z. H. Chen, H. Usta, Y. Zheng, Y. Xia, J. W. Hennek, R. P. Ortiz, T. J.Marks, A. Facchetti,J. Am. Chem. Soc.2013,135, 1986; c) N. Hergue,C. Mallet, G. Savitha, M. Allain, P. Frere, J.

Roncali,Org. Lett.2011,13, 1762; d) A. S. Ozen, C. Atilgan, G. Sonmez,J. Phys. Chem. C2007,111, 16362.

[17] a) M. Chen, L. Z. Li, H. Q. Wu, L. X. Pan, S. W. Li, B. R. He, H. K.Zhang, J. Z. Sun, A. J. Qin, B. Z. Tang,ACS Appl. Mater. Interfaces2018,10, 12181; b) H. K. Zhang, J. K. Liu, L. L. Du, C. Ma, N. L. C.Leung, Y. L. Niu, A. J. Qin, J. Z. Sun, Q. Peng, H. H. Y. Sung, I. D.Williams,R.T.K.Kwok,J.W.Y.Lam,K.S.Wong,D.L.Phillips,B.Z.Tang,Mater. Chem. Front.2019,3, 1143.

[18] a) W. F. Lai, W. T. Wong,Trends Biotechnol.2018,36, 713; b) W. F. Lai,M. C. Lin,J. Controlled Release2009,134, 158; c) W. F. Lai, M. C. Lin,Curr. Gene Ther.2015,15, 472.

[19] L. Pastor-Pérez, Y. Chen, Z. Shen, A. Lahoz, S. E. Stiriba,Macromol.Rapid. Commun.2007,28, 1404.

[20] R. Q. Ye, Y. Y. Liu, H. K. Zhang, H. F. Su, Y. L. Zhang, L. G. Xu, R. R. Hu, R. T. K. Kwok, K. S. Wong, J. W. Y. Lam, W. A. Goddard, B. Z. Tang, Polym. Chem.2017,8, 1722.

[21] a) L. Rincon, R. Almeida, D. Garcia-Aldea, H. D. Y. Riega,J. Chem.Phys.2001,114, 5552; b) Z. Y. Zhang, D. H. Li, W. R. Jiang, Z. G.Wang,Adv. Phys. X2018,3, 1428915.

[22] M. D. Eqbal, V. Gundabala,J. Colloid Interface Sci.2017,507,27.

[23] a) D. Y. Tu, D. Nilsson, R. Forchheimer,J. Disp. Technol.2013,9, 755;b) A. Aslund, A. Herland, P. Hammarstrom, K. P. R. Nilsson, B. H.Jonsson, O. Inganas, P. Konradsson,Bioconjugate Chem.2007,18,1860.

[24] W. F. Lai, H. C. Shum,ACS Appl. Mater. Interfaces2015,7, 10501.

[25] a) M. M. Amiji,Colloids Surf., B1998,10, 263; b) S. Hi-rano, M. Zhang, M. Nakagawa, T. Miyata,Biomaterials2000,21,997.

[26] A. L. Harkins, S. Duri, L. C. Kloth, C. D. Tran,J. Biomed. Mater. Res. B2014,102, 1199.

[27] a) J. A. Brenes-Salazar,Can. J. Physiol. Pharmacol.2015,93, 863; b)N. Garrido-Mesa, A. Zarzuelo, J. Galvez,Br. J. Pharmacol.2013,169,337; c) P. Joseph, J. Ponnaiya, M. Das, V. S. Chaitanya, S. Arumugam,M. Ebenezer,Indian J. Leprol.2016,88, 147; d) M. Katsuura, K. Ikeda,K. Mikajiri, M. Oishi, Y. Arakawa, K. Kataoka, N. Kurokawa,Med. DrugJ.2002,38, 1067.

[28] J. N. Minuth, T. M. Holmes, D. M. Musher,Antimicrob. AgentsChemother.1974,6, 411.

[29] J. H. Sung, M. R. Hwang, J. O. Kim, J. H. Lee, Y. I. Kim, J. H. Kim, S.W. Chang, S. G. Jin, J. A. Kim, W. S. Lyoo, S. S. Han, S. K. Ku, C. S.Yong, H. G. Choi,Int. J. Pharm.2010,392, 232.

[30] a)Clinical and Pathological Aspects of Skin Diseases in Endocrine,Metabolic, Nutritional and Deposition Disease(Eds:B.R.Smoller,F.Rongioletti), Springer, New York2010, p. 171; b) A. R. Bowen, T. H.McCalmont,J. Am. Acad. Dermatol.2007,57, 836.Adv. Healthcare Mater.2021,10, 2001267© 2020 Wiley-VCH GmbH2001267 (9 of 9)