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2020년 2월

석사학위논문

# 산화다공성실리콘 나노입자를 함유한 하이드로겔 고분자의 레보플록사신 약물전달시스템

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# 산화다공성실리콘 나노입자를 함유한 하이드로겔 고분자의 레보플록사신 약물전달시스템

Oxidized Porous Silicon Nanoparticles Covalent-Bonded with Levofloxacin in Hydrogel Polymer as a Drug Delivery System

2020년 2월 25일

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지도교수 손 홍 래

이 논문을 이학석사학위신청 논문으로 제출함.

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# 김하진의 석사학위논문을 인준함

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# ABSTRACT

## 산화다공성실리콘 나노입자를 함유한 하이드로겔 고분자의 레보플록사신 약물전달시스템

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하이드로겔 중합체에서 레보플록사신과 공유결합 된 산화다공성실리콘(OPS) 나노입자를 제조하여 약물전달의 효율을 측정한다. 레보플록사신은 촉매에 의해 OPS 나노입자의 Si-OH 표면에 공유결합을 형성한다. 입자의 평균 크기는 약 100 nm이다. 레보플록사신은 형광 특성을 갖기 때문에, 레보플록사신의 방출은 형광 분광계에 의해 측정되었다. OPS 나노입자로부터의 레보플록사신 방출량은 pH 7 수용액에서 시간에 따라 455 nm 에서 측정된다. 방출 프로파일의 분석은 레보플록사신(Levo-OPS)과 공유결합 된 OPS가 약물방출에 대한 큰 가능성을 나타낸다는 것을 발견했다. 약물방출의 속도는 OPS 나노입자 표면으로부터 실릴에스테르의 가수 분해에 의존한다.

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# 1. INTRODUCTION

Over the past decades, the development of nanotechnologies in designing pharmaceutical drugs for various diseases has been strongly affecting the biomedicine, both in diagnostics and therapy. Since nanotechnology offers a suitable means of site-specific and/or time-controlled delivery of drugs and other bioactive agents, the various nanocarriers such as nanoparticles, nanocapsule, micellar systems, and conjugates have been exploded for the potentials of a drug delivery system. Recently the design of biomaterials aims to improve drug delivery by controlling the timing and location of drug release. Ocular diseases affect 80 million people nowadays. Since million people wear contact lenses, there has been a great deal of interest in using contact lenses as the delivery device. The advances of biomaterials are being adapted to ocular drug delivery systems that may improve patient adherence through the use of therapeutic contact lenses. Although there are a variety of nonsurgical treatment options, drug-loaded contact lenses, are preferred treatment method. Soft contact lenses are hydrogels, water-soluble polymers that are cross linked to form networks. Hydrogels have a tremendous number of biomedical applications including drug delivery. However, water-soluble drugs, such as glaucoma, tend to elute very quickly from the highly hydrated polymer networks. The contact lenses made through molecular imprinting show fast release profiles than that of embedded microparticles. One of the greatest challenges with using hydrogels for drug delivery is that the contact lenses made through a covalent loading of drug into nanoparticles would maintain a local therapeutic dosage for a longer time. Utilizing these types of therapeutic contact lenses would lead to lower required dosage and less frequent drug application, thereby improving

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patient adherence. The purpose of this work is to test the feasibility of covalent loading of drug into porous silicon (PS) nanoparticles.

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# 2. EXPERIMENTAL DETAILS

## 2.1 Preparation of Porous Silicon

Porous silicon were prepared by electrochemical etching of a (100)-oriented p++-type silicon wafer (boron-doped, 0.8-1.2 mΩ cm, Siltronix). The electrochemical etch was conducted in Teflon cell with a platinum counter electrode. The applied current was supplied by a Keithley 2420 high-precision constant current source meter. The electrochemical etching was carried out by application of a constant current density of 200 mA/cm2 for 150 s in a 3:1 by volume mixture of absolute ethanol (Sigma Aldrich) and aqueous 48% HF (ACS reagent) was used as the etching solution.

## 2.2 Covalent Loading of Drug

PS sample and 5 mg of levofloxacin (Sigma Aldrich) were placed in 5 mL of DI water at room temperature for 24 h. PS was slowly oxidized, chemically functionalized, and covalently bonded with levofloxacin. For the preparation of oxidized porous silicon (OPS) covalently bonded with levofloxacin, PS was heated at 100oC in furnace chamber (HQ-DMF 14, Coretech) for 24 hours.

## 2.3 Preparation of OPS Nanoparticles Covalently Bonded with Drug; Levo-OPS Nanoparticles

Levo-OPS was placed in DI water in the presence of HF catalyst and fractured into multi-sized particles by sonication for overnight. The solution containing Levo-OPS nanoparticles were then centrifuged at 9600 rpm for 30 min. The supernatant containing Levo-OPS nanoparticles was re-centrifuged at 14000 rpm for 30 min. After the removal of the supernatant, the precipitates, Levo-OPS nanoparticles, were dried at 100 oC in furnace for 1 h.

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### 2.4 Quantification of Drug in Levo-OPS Nanoparticles

0.5 mg of Levo-OPS nanoparticles were incubated in a 0.3 M HCl 70% ethanol solution for overnight. The released quantities of levofloxacin to obtain a standard curve were measured by using fluorescence spectrometer.

### 2.5 Preparation of Levo-OPS Nanoparticle Lens and Drug Release Measurement

0.25 mg of Levo-OPS nanoparticles, volume mixture of 99.6:0.4 of 2-hydroxyethyl methacrylate; HEMA (Sigma Aldrich) and ethyleneglycol dimethacrylate; EGDMA (Thermo Fisher), and 0.1 wt% of azobisisobutyronitrile; AIBN (Sigma Aldrich) were mixed. 0.2 mL of mixture solution was dropped in the mold and heated for 6 h at 100 oC in furnace to cure. The cured Levo-OPS nanoparticles lens was immersed in DI water for hydration and transferred a beaker with 100 mL of phosphate-buffered saline (PBS) solution for drug release measurement. The drug release was measured by fluorescence spectrophotometer (F-7000, Hitachi) and the concentration of levofloxacin was calculated from the standard curve of known levofloxacin concentrations.

### 2.6 Preparation of levofloxacin-embedded lens (Levo-lens) and levofloxacin-embedded OPS (Levo-OPS)

For Levo-lens, 10 mg of levofloxacin, volume mixture of 99.6:0.4 of HEMA and EGDMA, and 0.1 wt% of AIBN were mixed. 0.2 mL of mixture solution was dropped in the mold and heated for 6 h at 100 oC in furnace to cure.
For Levo-OPS, PS sample and 5 mg of levofloxacin were placed in 5 mL of hexane at room temperature for 24 h. The levofloxacin-embedded PS was heated at 100oC in furnace chamber for 24 h and transferred a beaker with 100 mL of PBS solution for drug release measurement.

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## 2.7 Particle Size Analysis for Levo-OPS Nanoparticles

An average particle size of Levo-OPS nanoparticles was obtained by using dynamic light scattering (DLS-8000HL, Otsuka Electronics). Cold field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi) used to determine particles size and to obtain the morphology.

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# 3. RESULT AND DISCUSSION

Acute bacterial conjunctivitis, which requires an immediate management, is a prevalent infection. Generally a treatment with ocular antibiotics, levofloxacin, is recommended for bacterial conjunctivitis. Levofloxacin, which is a third generation of fluoroquinolone antibiotic, shows good activity against Staphylococcus aureus on cornea and conjunctiva. To develop a prolonged ocular drug delivery system with less frequency dosing, nanoparticles covalently bonded with levofloxacin in contact lenses will be a good candidate.

Figure 1 shows the schematic diagram for the preparation of Levo-OPS nanoparticles. It is well known that OPS nanoparticles are a biodegradable materials.6 The OPS was prepared by electrochemical etching of single-crystal silicon wafers in ethanolic HF solution. Levofloxacin was attached to the Si-OH surface of OPS in the presence of catalytic HF to form silyl ester (Figure 2).

In order to confirm the silyl ester, Fourier Transform Infrared (FTIR) spectra were obtained. Figure 3 shows the non-derivatized OPS (red line) and levo-derivatized OPS (blue line) spectra for the formation of the silyl ester group on the surface of OPS. The silyl ester carbonyl peak (Si-O-CO-R) at 1710 cm-1 and C=C peak from levofloxacin at 1600 cm-1 were obtained. Levo-OPS nanoparticles were prepared by ultrasonication of the Levo-OPS. Figure 4, obtained by dynamic light scattering measurements shows the size of Levo-OPS nanoparticles. Average diameter of Levo-OPS nanoparticles was about 100 nm. The surface morphology and size of Levo-OPS nanoparticles were obtained with cold FE-SEM and shown in Figure 5. The Levo-OPS nanoparticles appear spherical and fairly uniform with a well-defined micro- and meso-porous nanostructure. The pore diameters are of the order of 5-10 nm. Size of Levo-OPS nanoparticles was about 100 nm. The surface image of Levo-OPS nanoparticles indicated that the pore size of particle retained good porosity without destruction of porous

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structure during the ultrasonication. Since levofloxacin molecule has a strong fluorescence property, the quantity of levofloxacin release in OPS nanoparticles are obtained by using a standard curve of fluorescence measurement and was 0.024 mg of levofloxacin per 0.5 mg of Levo-OPS nanoparticles. The polyHEMA contact lens shows an average visible light transmittance (400-700 nm) of 93.8%.19 The Levo-OPS nanoparticle lens shows an average of 89.0%. The addition of 0.1% Levo-OPS nanoparticle lens does not lower this average transmission by more than 10%. Figure 6 shows PL spectra for the release of levofloxacin from Levo-OPS nanoparticle lens. Fast release of levofloxacin from contact lenses is observed within one day. Recently the mechanism for the hydrolysis of the silyl ester bond shown in Figure 7 is reported and indicated that the results reveal biexponential rate law consisting of a slow process and a fast process.22 Our result is very similar to their report. The release of levofloxacin was studied in three different ways: Levo-OPS nanoparticle lens, levofloxacin-embedded lens (Levo-lens), and levofloxacin-embedded OPS (Levo-OPS) nanoparticles. In the levofloxacin release profile, Levo-lens and Levo-OPS nanoparticle, shown in Figure 8(a), exhibit a burst release within 1 h. However Levo-OPS nanoparticle lens exhibits two step release processes: a fast release for 5 h and then a slow release. Figure 8(b) shows that the cumulative drug release from the Levo-OPS nanoparticle lens is 9.0 μg over 200 h, which is slower than that from molecular imprinting method and nanogel method.19 Sailor et al. reported that OPS degraded into silicic acid in a short time and dissolve in PBS solution. However, degradation is slowed by addition of a polymeric coating.6 Silicon is a common trace element in humans and a biodegradation product of OPS, orthosilicic acid ($Si(OH)_4$), is the form absorbed by humans and is naturally found in tissues. These results demonstrate a new type of drug delivery system with a low-toxicity degradation pathway.

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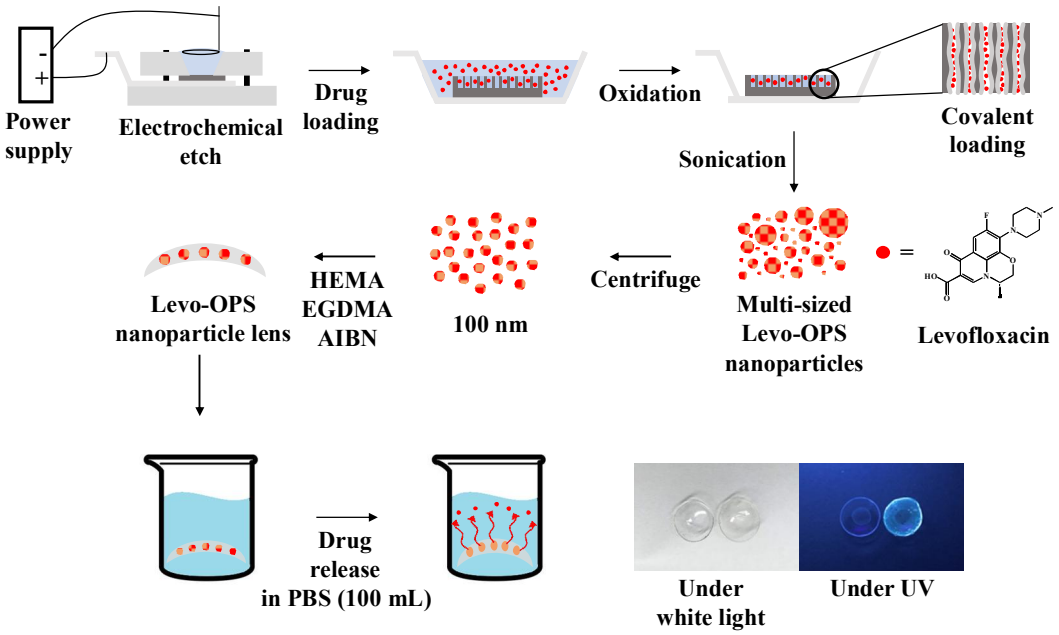


Fig. 1. Schematic diagram for the preparation of Levo-OPS nanoparticles.

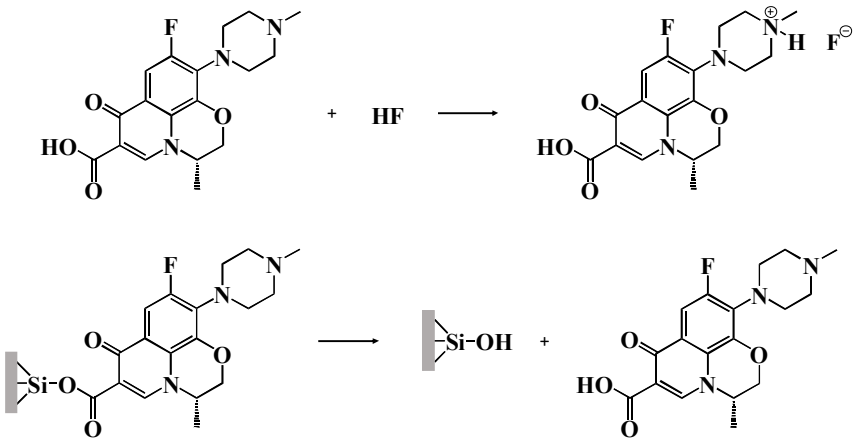


Figure. 2. Surface derivatization of OPS with levofloxacin.

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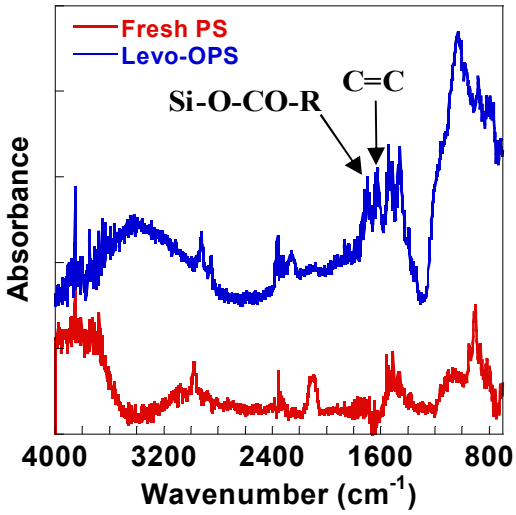


Figure. 3. FTIR spectra for OPS and Levo-OPS.

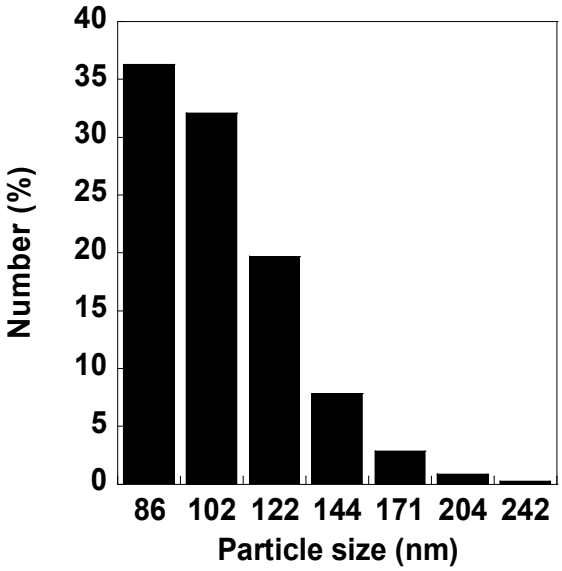


Figure. 4. DLS data for Levo-OPS nanoparticles.

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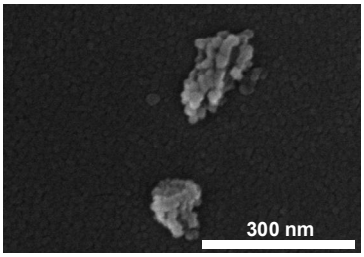


Figure. 5. FE-SEM image of Levo-OPS nanoparticles.

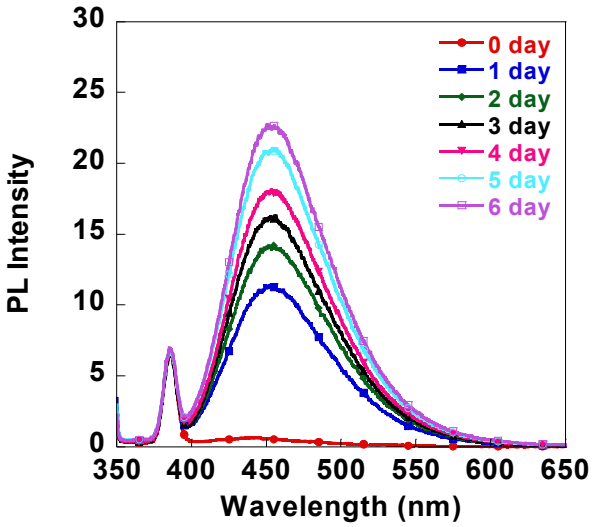


Figure. 6. PL spectra upon the release of levofloxacin from Levo-OPS nanoparticle lens.

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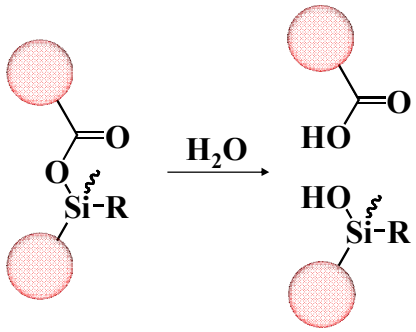
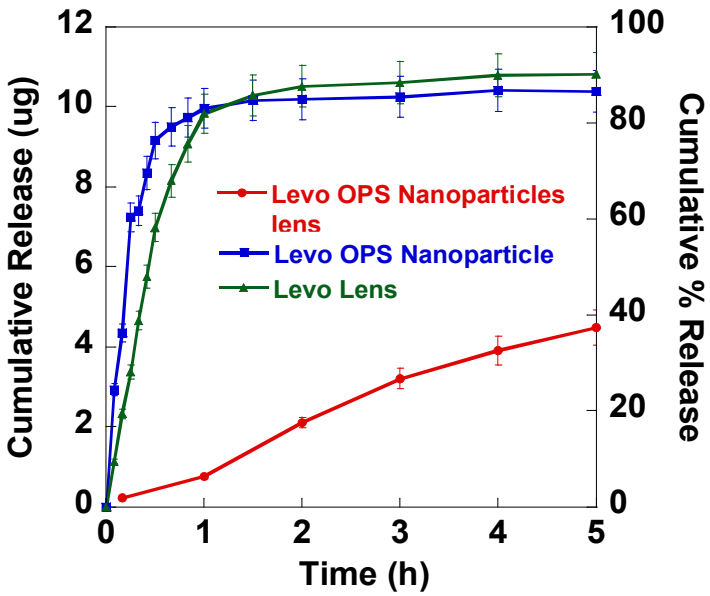



Figure. 7. Hydrolysis of silyl ester.



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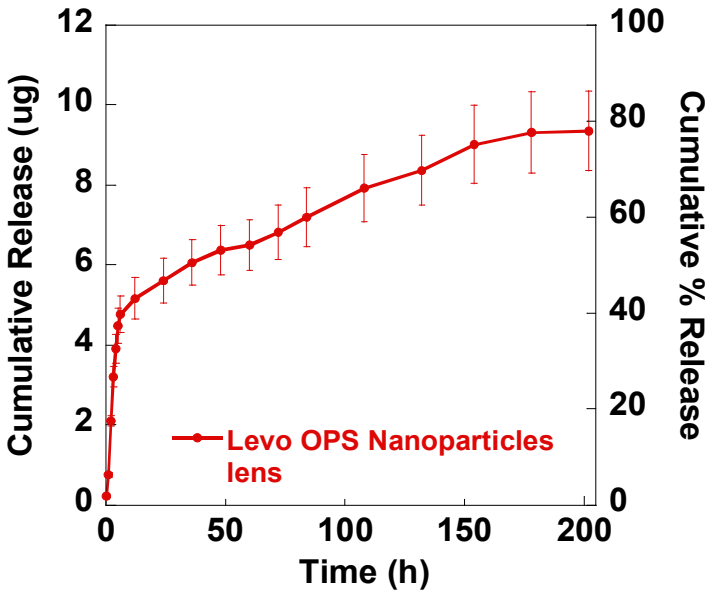


Figure. 8. Drug release profile for Levo-OPS nanoparticle lens, Levo-lens, and Levo-OPS nanoparticle in PBS for 5 h and 200 h.

# 4. CONCLUSION

These studies have demonstrated that the levofloxacin covalent-bonded contact lens serves as an effective device for the hydrolysis-triggered delivery of levofloxacin. The Levo-OPS nanoparticle lens subsequently allows sustained drug release due to the hydrolysis of silyl ester. This eliminates the uncontrolled release observed with other lens types. Optical clarities maintained at practical levels. This work has provided a new method for preparing contact lenses.

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# References

1. D. A. LaVan, T. McGuire, and R. Langer, Nat.Biotechnol. 21, 1184 (2003).
2. B. Ziaie, A. Baldi, M. Lei, Y. Gu, and R. A. Siegel, Adv. Drug Deliv. Rev. 56, 145 (2004).
3. L. M. Bonanno and L. A. DeLouise, Biosens. Bioelectron. 23, 444 (2007).
4. K. S. Soppimath, T. M. Aminabhavi, A. R. Kulkarni, and W. E. Rudzinski, J. Control. Release 70, 1 (2001).
5. E. J. Anglin, L. Cheng, W. R. Freeman, and M. J. Sailor, Adv. Drug Deliv. Rev. 60, 1266 (2008).
6. J.-H. Park, L. Gu, G. V. Maltzahn, E. Ruoslahti, S. N. Bhatia, and M. J. Sailor, Nat. Mater. 8, 331 (2009).
7. C. E. Mora-Huertas, H. Fessi, and A. Elaissari, Int. J. Pharm. 385, 113 (2010).
8. Y. Chen, H. Chen, D. Zeng, Y. Tian, F. Chen, J. Feng, and J. Shi, ACS Nano 4, 6001 (2010).
9. R. Savić, L. Luo, A. Eisenberg, and D. Maysinger, Science 300, 615 (2003).
10. A. Rösler, G. W. M. Vandermeulen, and H.-A. Klok, Adv. Drug Deliv. Rev. 64, 270 (2012).
11. S. Aryal, C.-M. J. Hu, and L. Zhang, ACS Nano 4, 251 (2010).
12. P. Huang, D. Wang, Y. Su, W. Huang, Y. Zhou, D. Cui, X. Zhu, and D. Yan, J. Am. Chem. Soc. 136, 11748 (2014).
13. S. Castleberry, M. Wang, and P. T. Hammond, ACS Nano 7, 5251 (2013).
14. X. Su, B. S. Kim, S. R. Kim, and P. T. Hammond, and D. J. Irvine, ACS Nano 3, 3719 (2009).
15. H. A. Quigley and A. T. Broman, Br. J. Ophthalmol. 90, 262 (2006).
16. J. B. Ciolino, T. R. Hoare, N. G. Iwata, I. Behlau, C. H. Dohlman, R. Langer and D. S. Kohane, Invest. Ophthalmol. Visual Sci. 50, 3346 (2009).
17. J. P. Bertram, S. S. Saluja, J. McKain, and E. B. Lavik, J. Microencapsul. 26, 18 (2009).
18. H. Hiratani, A. Fujiwara, Y. Tamiya, Y. Mizutani, and C. Alvarez-Lorenzo, Biomaterials 26, 1293 (2005).
19. H. J. Kim, K. Zhang, L. Moore, and D. Ho, ACS Nano 8, 2998 (2014).

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20. H. Gupta1, M. Aqil, R. K. Khar, A. Ali1, A. Bhatnagar, and G. Mittal, J. Drug. Target. 19, 409 (2011).
21. M. Plehiers, US Patent, 10/510,369, (2006).
22. W. S. Schmidt, P. Filippov, A. Kersch, M. K. Beyer, and H. Clausen-Schaumann, ACS Nano 6, 1314 (2012).