Development of silver nanoparticle-based hydrogel composites for antimicrobial activity

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ABSTRACT

Antimicrobial function of Ag nanoparticles (NPs) has a strong correlation with the released Ag+ cations that are produced by oxidation of Ag NPs in a solution state under ambient condition. Therefore, in order to develop anti-infective materials for biomedical applications, one needs to include Ag NPs inside biocompatible materials, which can allow slow release of Ag+ cations. Hydrogels of natural polymers could be an ideal choice for the purpose because (a) the physicochemical properties of hydrogels resemble with biological tissue, and (b) the inclusion of Ag NPs inside hydrogels prevents the direct release of Ag NPs, while allowing the release of Ag+ cations out of the hydrogels. In this regard, we present a simple strategy for producing Ag NPs-containing hydrogel based on natural alginate polymers. The chemical modification of alginate, blending with Ag NPs, gelation by photo-crosslinking process have been discussed in connection with antimicrobial reaction on model bacterium.

ARTICLE HISTORY

Received 28 November 2018
Accepted 20 January 2020

KEYWORDS

Hydrogels; nanoparticles; alginate; antimicrobial activity

1. Introduction

Hydrogels are 3-dimensionally interconnected hydrophilic polymers, which can absorb an extensive amount of water within the structures without being dissolved in water. Since the chemical, electrical, and mechanical properties of polymer hydrogels could be quite analogous with those present in biological tissues, there have been extensive efforts to utilize the hydrogels in biological applications such as drug delivery (1–12), wound/burn dressing (13, 14), scaffolds for tissue engineering (15–17), and others (18–21). In particular, the inclusion of drugs inside polymer hydrogels may represent an innovative drug delivery system because the release rate of the loaded drugs can be regulated by external stimuli (1, 2, 4–9). In many cases, the change in pH (2, 3), temperature (1, 7, 9), and light (4) has triggered the swelling, shrinkage, or degradation of the hydrogels to release the drugs in a controlled way. Alternatively, in the absence of the external stimuli, the slow diffusion nature of the drug release can ensure a constant delivery as well as prolonged duration of drugs, which is necessary for exerting long-term efficacy (10–12).

In this regard, antimicrobial agents are an important class of drugs that can take advantage of polymer hydrogels. In particular, the inclusion of silver nanoparticles (Ag NPs) as antimicrobial agents and/or an alternative to antibiotics (15) has been suggested as an interesting approach in developing anti-infective materials. For the case of colloidal Ag NPs, it has been reported that the formation of Ag+ cations from Ag NPs by oxidation reaction would be responsible for the antimicrobial activity because Ag+ cations had been found to disrupt the bacterial cell membranes, and also inhibit enzymatic activity of bacterial cells (22–25). Additionally, Ag NPs themselves possess antimicrobial activity; however, they could also exert dermal toxicity (26). Therefore, it is highly desirable to isolated Ag NPs from the infected area, while supplying Ag+ cations to the target area. In this circumstance,
hydrogel containing Ag NPs may address the issue; the cross-linked polymer network can prevent the release of Ag NPs from the gels, while allowing the release of Ag⁺ cations that are produced by oxidation of Ag NPs inside the hydrogel.

To prepare Ag NPs-based hydrogel composites, one can utilize either in-situ or ex-situ approach to introduce NPs inside hydrogels. In the case of in-situ approach, hydrogels were first prepared after cross-linking polymers, onto which Ag⁺ cations (a precursor of Ag NPs) were coordinated or complexed with specific chemical groups in the constituent polymers (27, 28). Since the binding of Ag⁺ to the hydrogels occurs in a solution state, the subsequent reduction of Ag⁺-loaded gel by chemical reagent ensures the homogeneous NP synthesis within hydrogels. However, the utilization of reduction agent such as NaBH₄ could potentially exert cytotoxicity. For the ex-situ approach, pre-synthesized Ag NPs have been mixed with the constituent polymers before cross-linking polymers. During cross-linking process, however, the agglomeration of NPs potentially occurs, which could substantially reduce the antimicrobial activity of Ag NPs. Since the stability of colloidal NPs is an important parameter, the surface of Ag NPs has been modified by collagen (15) and human serum albumin (22) before gel formation.

In this study, we prepared alginate-based hydrogels containing ex-situ synthesized Ag NPs. Alginate is a linear unbranched polysaccharide derived from brown algae, and forms hydrogel upon the addition of divalent cations such as Ca²⁺ because the carboxylic acid moiety in alginate backbone interacts with the counter cations. However, the direct utilization of cation-assisted crosslinking method would encounter NP agglomeration because the colloidal stability of Ag NPs became weak in the high ionic concentration. To overcome this issue, we introduced methacrylate group on the alginate backbone with well-known carbodiimide chemistry (17). The methacrylated alginates underwent photo-crosslink reaction in the presence of Ag NPs, which resulted in the formation of alginate hydrogel with homogenous NP distribution. The antimicrobial activity of prepared hydrogel composites was tested against Escherichia coli (E. coli), which exhibited dose-dependent inhibition on bacterial growth.

2. Experimental section

2.1. Materials

Alginic acid sodium salt, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), 2-aminoethyl methacrylate (AEMA), 2-morpholinoethanesulfonic acid (MES), sodium chloride (NaCl, 99% pure), Irgacure D2959 (photoinitiator), silver nitrate (>99.9% pure), sodium borohydride (NaBH₄, >99% pure), and trisodium citrate (SC, 99% pure) were purchased from Sigma-Aldrich and used as received without further purification.

2.2. Synthesis of Ag NPs

Ag NPs were prepared according to a literature (29). Briefly, an aqueous solution (192 mL) containing sodium borohydride (0.015 g) and trisodium citrate dehydrate (0.099 g) were heated at 60°C for 30 min under vigorous stirring. Subsequently, AgNO₃ (8 mL, 1.17 mM) was added dropwise into the reaction medium and the temperature of the solution mixture was set to 90°C. Then, pH of the solution was adjusted to 10.5 using NaOH (0.1 M). The heating was continued for an additional 20 min, and the reaction mixture was cooled down to room temperature. The resulting NP solution was further centrifuged (9000 rpm, 40 min) and re-dispersed in deionized water. This washing step was repeated twice and the resulting aqueous Ag NPs were kept in the dark at 4°C for future use.

2.3. Synthesis of methacrylated alginate

To fabricate hydrogel, we modified the chemical structure of alginate with methacrylate group according to the literature method (17). Briefly, alginic acid sodium salt (4 g) was dissolved in 50 mM MES buffer solution (1% w/v, pH 6.5) containing 0.5 M NaCl. Then, NHS (1.06 g) and EDC (3.5 g) were added to the solution. The solution was stirred for 5 min. Next, AEMA (1.52 g) was further added to the solution, and the mixture was left at room temperature for 24 h. The polymer in the resulting solution was precipitated by adding an excess amount of acetone, dried under vacuum and rehydrated to a 1% w/v solution in deionized water. The resulting solution was further purified by dialysis against deionized water for 3 days and lyophilized to obtain methacrylated alginate.

2.4. Preparation of hydrogel

Photo-crosslinked hydrogel was prepared by dissolving methacrylated alginate (0.08 g) and Irgacure D2959 (0.002 g) in the aqueous solution of Ag NPs (4 mL) under stirring. Next, the reaction mixture was poured into Teflon molds (PTFE, diameter = 30 mm) and irradiated with 365 nm UV light (6 W, Vilber Lourmat) for 15 min to form the hydrogels. The pure alginate
hydrogel without Ag NPs was also prepared after adding the same amount methacrylated alginate and Irgacure D2959 to dionized water.

2.5. Antimicrobial activity assay

To evaluate the antimicrobial effect, we used spread plate method. Spread plate method is typically used to counts the number of colonies over the surface of an agar plate. The *Escherichia coli* KCTC 1116 strain was cultured in Luria–Bertani (LB) broth until the absorbance reached 0.8 at 600 nm of optical density. In order to investigate the antimicrobial activity of the hydrogel composite against *E. coli*, 1 mL of culture broth was inoculated in 29 mL of PBS solution (pH 7.4). Then, the hydrogel with and without Ag NPs was placed in the mixture solution of *E. coli* and PBS. At this time, the concentration of hydrogel in the mixture solution was adjusted to 1g/mL. The antimicrobial reaction was performed at 37°C for 24 h. After the reaction, the reactants were diluted to different degrees (down to 10^{-7} folds), and 100 µL of aliquots was smeared on the LB agar. The plates were incubated at 37°C for 24 h. After the incubation, the number of colony was counted and converted to colony-forming unit (CFU/mL). In addition, compared with control group to verify the efficiency of antimicrobial activity.

2.6. Characterization

UV−Vis absorbance data were obtained on a Carry 8454 UV-Vis spectrophotometer (Agilent technologies). Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS-90 (Malvern Instrument). Transmission electron microscopy (TEM) was carried out with the Hitachi H-7500 instrument operating at 80 kV. ¹H NMR spectra were recorded on a JNM-ECP 600 (JEOL instrument).

3. Results and discussion

Since the functionality of hydrogel composite can be engineered by the inclusion of an appropriate NPs, we synthesized Ag NPs by a conventional citrate-reduction method to produce antimicrobial hydrogels. As shown in the UV-Vis spectrum (Figure 1(a)), the prepared Ag NPs have an absorption peak at ∼400 nm that can be assigned as localized surface plasmon resonance of Ag NPs. The symmetric shape of the plasmonic peak of Ag NPs can imply the homogeneity of NPs in their size and shape. From TEM image, spherical Ag NPs were clearly discernible with relatively narrow size distribution. For better examination of the size, DLS spectrum of the NP solution was analyzed as shown in Figure 1(b). After fitting DLS result with Gaussian function (red solid line), the average diameter of Ag NPs was determined as ∼12 nm. It needs to be noted that the synthesized Ag NPs are quite stable in a solution state without noticeable agglomeration, which can be ascribed to the negatively charged citrate molecules on the surface of NPs. (zeta potential = −39.9 mV). Therefore, the agglomerated NPs in the TEM images were produced by solvent evaporation during TEM sampling.

As a counterpart to Ag NPs, we employed alginate for the formation of hydrogel. In principle, the simple addition of divalent cations such as Ca^{2+} can induce cross-linking of alginate backbone by coordination with carboxylic acid group (confer the molecular structure in Figure 2(a)). However, the addition of ions to citrate-stabilized Ag NPs can induce NP agglomeration by reducing electrical static repulsing among NPs. For this reason, we utilized photo-crosslinking for the gel formation. To this end, the alginate molecules were reacted with 2-aminoethyl methacrylate (AEMA) by EDC-NHS chemistry (Figure 2(a)). The introduction of methacrylate group in the alginate backbone can be subject to photo-crosslinking process in the presence of Ag NPs. The chemical modification of alginate

Figure 1. (a) UV-Vis spectra of Ag NPs. The inset is TEM image of the Ag NPs. (b) Size distribution of the Ag NPs.
backbone was verified by comparing $^{1}H$ NMR spectra of alginate and methacrylated alginate. As shown in Figure 2(b), new peaks have appeared at δ6.1, 5.7 (denoted as i) and 1.9 (denoted as ii), each of which can be assigned to proton peaks of vinyl methylene and methyl in the methacrylate group ($^{17}$).

Next, we attempted to produce hydrogels by blending methacrylated alginate and Ag NPs. In this step, we first adjusted the concentration of Ag NPs in the aqueous solutions to study dose-dependent antimicrobial activity of the final gels. To quantify the amount of Ag NPs, the absorbance values of Ag NPs in the solutions were adjusted to 0.5, 1.5, and 2.5 as shown in Figure 3(a). As well known, the absorbance (A) of Ag NPs is related to molar concentration (C), molar extinction coefficient (ε), and beam path length ($b = 1 \text{ cm}^{-1}$) by Lamber-Beer law of $A = \varepsilon bC$. From the literature, the molar extinction coefficient of Ag NPs (diameter = 12 nm) was found to be $10.1 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$ ($^{21}$). By inserting this value into Lamber-Beer law, the molar concentration of Ag NPs was calculated as ca. 0.5, 1.5, and 2.5 nM. To each NP solutions (4.0 mL), we added methacrylated alginate (0.08 g) and photoinitiator (0.002 g). Then, the reaction mixture was poured into Teflon mold having a diameter...
of 30 mm. Here, it needs to be noted that both citrate-stabilized Ag NPs and methacrylated alginate are negatively charged by carboxylic acid groups. Therefore, they formed homogenous mixtures without any noticeable agglomeration. The reaction mixture was illuminated by UV light to initiate photo-crosslinking process. We also prepared pure hydrogel without Ag NPs by simply adding the same amount of methacrylated alginate and photoinitiator to deionized water. Figure 3(b–e) are photos of the prepared hydrogels with different amount of Ag NPs. It can be confirmed that the distribution of Ag NPs inside each hydrogels is quite uniform and the yellow colors of hydrogel, which is caused by the presence of Ag NPs, became more intense with the NP concentration.

Confirming the formation of homogenous Ag NPs-containing hydrogels, we tested their antimicrobial activity. To this end, *E. coli* strain was cultured in LB broth until the absorbance reached 0.8 at 600 nm. Then, the prepared hydrogels (Figure 3(b–e)) and cultured solution were reacted at 37°C for 24 h. After antimicrobial reaction, the reactants were diluted in a various concentration, and 100 µL of aliquots was smeared on the LB agar. The plates were incubated at 37°C for 24 h, and utilized for the measurement. As shown in Figure 4, the pure hydrogel without Ag NPs did not exert meaningful antimicrobial activity. There was only monotonic decrease of the white region, which is an indicative of bacteria colony, upon dilution. For the hydrogels with 0.5 nM of Ag NPs, quite similar trends have been observed. In the stark contrast, for the hydrogels with 1.5 and 2.5 nM of Ag NPs, we observed complete inhibition of bacterial growth from all the incubated plates. For a better comparison, colony-forming unit (CFU/mL) was evaluated from the cultured plates, and the result was summarized in Table 1.

It is interesting to note that the increase of NP concentration from 0.5 nM to 1.5 nM resulted in dramatic changes in the antimicrobial activity from the negligible effect on *E. coli* to complete inhibition of bacterial growth. To understand this behavior, it would be worthwhile to note that Ag NPs can be oxidized in aqueous solutions under air condition and release Ag⁺ cations, which contributes to antimicrobial activity of Ag NPs. On the other hand, the bactericidal effect of Ag NPs themselves remains still elusive although recent study under an anaerobic condition revealed negligible antimicrobial effect from non-oxidized Ag NPs (30). In our study, since Ag NPs were entrapped inside the cross-linked alginites, they would not be diffused out from the hydrogels. This ruled out the possibility of the additional bactericidal effect of NPs themselves. Therefore, the release of Ag⁺ cations from hydrogel could be solely responsible for the observed result. When the concentration of Ag NPs was too low, however, the release of produced Ag⁺ cations into cultured medium would be restricted by the favorable interaction between Ag⁺ cations and –COO⁻ group in alginate backbone. With increasing the concentration of Ag NPs, there would be an excess amount of Ag⁺ cations that can be released into the reaction medium. As a result, the hydrogel starts to exert its antimicrobial effect on the model bacterium (*E. coli*), which resulted in the inhibition of bacterial growth. We also noted that the antimicrobial activity of hydrogel composites was quite comparable with that of colloidal Ag NPs without alginate as shown in Table 2. Based on the discussion, the preparation of nano-/micro-sized hydrogels would facilitate the release of Ag⁺ cations by providing the larger surface area. However, the consumption of Ag⁺ cations with the fast release rate would eventually decrease the antimicrobial activity. Unlike metal NPs, some polymeric

| Table 1. Colony forming units evaluated from cultured plates with various alginate hydrogels. |
|-----------------------------------------------|
| **Concentration of Ag NPs** | **Bacterial counts (CFU/mL)** |
| | Initial | 10⁻¹ | 10⁻² | 10⁻³ | 10⁻⁴ | 10⁻⁵ | 10⁻⁶ | 10⁻⁷ |
| 0 nM | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC | 63 × 10⁹ | 96 × 10⁹ |
| 0.5 nM | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC | 24 × 10⁹ | 27 × 10⁹ |
| 1.5 nM | 12 × 10² | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.5 nM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Note: TNTC: Too numerous to count.

| Table 2. Colony forming units evaluated from cultured plates with hydrogel composites and colloidal Ag NPs. After antimicrobial reaction, the reactants were diluted to 10⁻⁶ fold. |
|-----------------------------------------------|
| **Concentration of Ag NPs** | **Bacterial counts (CFU/mL)** |
|-----------------------------------------------|
| **Hydrogel Composites** | 0.0 nM | 0.25 nM | 0.50 nM | 0.75 nM | 1.0 nM | 1.5 nM |
| 10 × 10⁹ | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 × 10⁹ | 1 × 10⁹ | 0 | 0 | 0 | 0 | 0 |
| **Colloidal Ag NPs** | 0.0 nM | 0.25 nM | 0.50 nM | 0.75 nM | 1.0 nM | 1.5 nM |
| 7 × 10⁹ | 0 | 0 | 0 | 0 | 0 | 0 |
particles could exert antimicrobial effect without the consumption problem (32, 33). In this regard, it would be better to exert long-term antimicrobial effect by controlling the release of Ag⁺ cations. Since the release rate of the drug in polymer gels can be engineered by the hydrophilicity of the gel matrix (33), the adjustment of chemical nature as well as physical structures could optimize the diffusion of Ag⁺ cations for effective antibacterial effect with prolonged efficacy.

4. Conclusion
In this study, we present a simple approach to prepare hydrogel composed of alginate and Ag NPs and their antimicrobial activity on a model bacterium (E. coli). For the homogenous NP dispersion in the hydrogel, we utilized photo-crosslinking method after modifying the chemical structure of polymer backbone with methacrylate group. The resulting methacrylated alginate was miscible with citrate-stabilized Ag NPs without agglomeration, which could be ascribed to the electrostatic repulsing between NPs and alginate molecules. Upon exposure to UV light, alginate hydrogels with different amount of Ag NPs were prepared, which exerted a strong bactericidal effect on E. coli. We further discussed that the released Ag⁺ cations out of hydrogels instead of NPs themselves would be the main toxicant for the observed bacterial death. Since many antimicrobial agents could be easily introduced in the hydrogel composites by simply mixing them with methacrylated alginate before photo-crosslinking, it is interesting to further study the bactericidal effect of hydrogel composites with more economically potent NPs such as copper. Overall, the hydrogel composites with metal NPs could find practical applications in biomedical area such as wound/burn dressing (13, 34, 35) and functional antimicrobial coatings (36) because they can provide a biocompatible environment with antimicrobial activity.

Funding
This study was supported by Open Laboratory Operational Business Developing and Diffusing the Regional Specialization Technology funded by the Busan Institute of S&T Evaluation and Planning (BISTEP). This work was supported by the BB21+ Project in 2019.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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Figure 4. Photographs of LB plates upon dilutions after the addition of alginate hydrogels having different concentrations of Ag NPs.
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References

[1] Wu, J.; Chen, A.; Qin, M.; Huang, R.; Zhang, G.; Xue, B.; Wei, J.; Li, Y.; Cao, Y.; Wang, W. Nanoscale. 2015, 7, 1655–1660.
[2] Gao, W.; Vecchio, D.; Li, J.; Zhu, J.; Zhang, Q.; Fu, V.; Li, J.; Thamphiwatana, S.; Lu, D.; Zhang, L. ACS Nano 2014, 3, 2900–2907.
[3] Abandansari, H.M.; Nabid, M.R.; Rezaei, S.J.T.; Niknejad, H. Polymer 2014, 55, 3579–3590.
[4] Shah, S.; Sasmal, P.K.; Lee, K–B J. Mater. Chem. B 2014, 2, 7685–7693.
[5] Servant, A.; Leon, V.; Jasim, D.; Methven, L.; Limousin, P.; Fernandez-Pacheco, E.V.; Prato, M.; Kostarelos, K. Adv. Healthcare Mater 2013, 4, 1334–1343.
[6] Servant, A.; Methven, L.; Williams, R.P.; Kostarelos, K. Adv. Healthcare Mater 2013, 2, 806–811.
[7] Cheng, Z.; Chai, R.; Ma, P.; Dai, Y.; Kang, X.; Lian, H.; Hou, Z.; Li, C.; Lin, J. Langmuir 2013, 29, 9573–9580.
[8] Campbell, S.B.; Patenaude, M.; Hoare, T. Biomacromolecules 2013, 14, 644–653.
[9] Wu, W.; Shen, J.; Banerjee, P.; Zhou, S. Biomaterials 2010, 31, 7555–7566.
[10] Nagahama, K.; Kawano, D.; Oyama, N.; Takemoto, A.; Kumano, T.; Kawakami, J. Biomacromolecules 2015, 16, 880–889.
[11] Zhong, D.; Liu, Z.; Xie, S.; Zhang, W.; Zhang, Y.; Xue, W. J. Appl. Poly. Sci. 2013, 129, 767–772.
[12] Kuang, H.; He, H.; Zhang, Z.; Qi, Y.; Xie, Z.; Jing, X.; Huang, Y. J. Mater. Chem. B 2014, 2, 659–667.
[13] Capanema, N.S.V.; Mansur, A.A.P.; Carvalho, S.M.; Mansur, L.L.; Ramos, C.P.; Lage, A.P.; Mansur, H.S. J. Appl. Poly. Sci. 2018, 135, 45812.
[14] Kumar, P.T.S.; Lakshmanan, V–K; Anilkumar, T.V.; Ramya, C.; Reshmi, P.; Unnikrishnan, A.G.; Nair, S.V.; Jayakumar, R. ACS Appl. Mater. Interfaces 2012, 4, 2618–2629.
[15] Alarcon, E.L.; Udekwu, K.J.; Noel, C.W.; Gagnon, L.B.;–; Taylor, P.K.; Vulesevic, B.; Simpson, M.J.; Gkotzis, S.; Islam, M.M.; Lee, C–J; Richter–DAHfors, A.; Mah, T–F; Suuronen, E.J.; Scaino, J.C.; Griffith, M. Nanoscale. 2015, 7, 18789–18798.
[16] Bonino, A.C.; SAmorezov, J.E.; Jeon, O.; Alsberg, E.; Khan, S.A. Soft Matter 2011, 7, 11510–11517.
[17] Jeon, O.; Bouhadir, K.H.; Mansour, J.M.; Alsberg, E. Biomaterials 2009, 30, 2724–2734.
[18] Lengert, E.; Parakhonskiy, B.; Khalenkow, D.; Zecic, A.; Vanghell, M.; Moreno, J.M.M.; Braeckman, B.P.; Skirtach, A.G. Nanoscale. 2018, 10, 17249–17256.
[19] Hou, C.; Ma, K.; Jiao, T.; Xing, R.; Li, K.; Zhou, J.; Zhang, L. RSC Adv. 2016, 6, 110799–110807.
[20] Otari, S.V.; Patil, R.M.; Waghmare, S.R.; Ghish, S.J.; Pawar, S.H. Dalton Trans. 2013, 42, 9966–9975.
[21] Zheng, Y.; Wang, A. J. Mater. Chem. 2012, 22, 16552–16559.
[22] Grade, S.; Eberhard, J.; Neumeister, A.; Wagener, P.; Winkel, A.; Stiesch, M.; Barckowski, S. RSC Adv. 2012, 2, 7190–7196.
[23] Hwang, E.T.; Lee, J.H.; Chae, Y.J.; Kim, Y.S.; Kim, B.C.; Sang, B–I; Gu, M.B. Small 2008, 4, 746–750.
[24] Pal, S.; Tak, Y.K.; Song, J.M. Appl. Environ. Microbiol. 2007, 73, 1712–1720.
[25] Sondi, I.; Salopek–Sondi, B. J. Colloid Interf. Sci. 2004, 275, 177–182.
[26] Chen, X.; Schluesener, H.J. Toxicol. Lett. 2008, 176, 1–12.
[27] Varaprasad, K.; Mohan, Y.M.; Ravindra, S.; Reddy, N.N.; Vimala, K.; Monika, K.; Sreedhar, B.; Raju, K.M. J. Appl. Polym. Sci. 2010, 115, 1199–1207.
[28] Mohan, Y.M.; Lee, K.; Premkumar, T.; Geckeler, K.E. Polymer 2007, 48, 158–164.
[29] Agnihotri, S.; Mukherji, S.; Mukherji, S. RSC Adv. 2014, 4, 3974–3983.
[30] Xiu, Z.; Zhang, Q.; Puppala, H.L.; Colvin, V.L.; Alvarez, P.J.J. Nano Lett. 2012, 12, 4271–4275.
[31] Paramelle, D.; Sadovoy, A.; Gorelik, S.; Free, P.; Hobley, J.; Fernig, D.G. Analyst 2014, 139, 4855–4861.
[32] Ozay, O.; Akcali, A.; Otkun, M.T.; Silan, C.; Aktas, N.; Sahiner, N. Colloids Surf. B. 2010, 79, 2629–266.
[33] Silan, C.; Akcali, A.; Otkun, M.T.; Ozsey, N.; Butun, S.; Ozay, O.; Sahiner, N. Colloids Surf. B. 2012, 89, 248–253.
[34] Fan, Z.; Liu, B.; Wang, J.; Zhang, S.; Ling, Q.; Gong, P.; Ma, L.; Yang, S. Adv. Func. Mater. 2014, 24, 3933–3943.
[35] Ghavaminejad, A.; Park, C.H.; Kim, C.S. Biomacromolecules 2016, 17, 1213–1233.
[36] Ahearn, D.G.; Grace, D.T.; Jennings, M.J.; Borzajani, R.N.; Boles, K.J.; Rose, L.J.; Simmons, R.B.; Ahanotu, E.N. Curr. Microbiol. 2000, 41, 120–125.