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ScienceDirect
Polymer 48 (2007) 158–164
polymer
www.elsevier.com/locate/polymer

Hydrogel networks as nanoreactors: A novel approach to silver nanoparticles for antibacterial applications

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Received 14 June 2006; received in revised form 18 October 2006; accepted 25 October 2006
Available online 22 November 2006

Abstract

Hydrogel networks based on *N*-isopropylacrylamide (NIPAM) and sodium acrylate (SA) were prepared by redox-polymerization in the presence of *N*,*N*′-methylenebisacrylamide (MBA). Highly stable and uniformly distributed silver nanoparticles have been prepared using these hydrogel networks as a carrier via in situ reduction of silver nitrate in the presence of sodium borohydride as a reducing agent. It has been demonstrated that the hydrogel hybrid with different sizes of silver nanoparticles can be effectively employed as antibacterial material.

Keywords: Poly(*N*-isopropylacrylamide); Silver nanoparticle; Hydrogel networks

1. Introduction

Nanoparticles (NPs) and nanostructured materials [1] are considered as an important class of materials that have found fascinated interest in biomedical [2], catalytical [3,4], optical [5], and electronic [6] as well as quantum-size domain [7] applications. A number of methods have been suggested and developed [1,8,9] for the synthesis of non-agglomerated nanoparticles. These include polymers [10,11], biological macromolecules [12,13], latex particles [14], mesoporous inorganic materials [15], dendrimers [16,17], microgels or hydrogels [18–22], colloidal systems [23,24], and others. Most of the above mentioned systems provide a good stability for nanoparticles, but microgel or hydrogel network template/carrier systems can act in a similar way. In addition, these make it more convenient for producing smaller size nanoparticles. However, these make it more convenient for producing smaller size nanoparticles. Recent research efforts are heading for exploiting the in situ synthesis of metal nanoparticles within the polymeric network architectures [25,26] and the synthesized products using these approaches are leading to new hybrid or composite systems in chemistry and engineering science. In fact, three-dimensional network hydrogels are appropriate templates [19] for producing nanoparticles over the conventional non-aqueous and polymeric systems for biomedical applications, in view of their exceptional compatibility with biological molecules, cells, and tissues. In this way the carrier system of, for example, dendrimers or microgels act as 'nanoreactors' that immobilize the particles and provide an easy handling.

Hydrogels offer large free space between the crosslinked networks in the swollen stage that can act as a nanoreactor for the nucleation and growth of the nanoparticles. A controlled Ag nanocluster deposition into the poly[(*N*-isopropylacrylamide)-*co*-(acrylic acid)-*co*-(2-hydroxylethyl acrylate)] microgel was already achieved [19]. Xu et al. [27] developed a hybrid Ag–microgel for photonic crystals and studied their thermosensitive characteristics. Pich et al. [28] described the synthesis and characterization of hybrid microgels consisting of poly[(vinylcaprolactam-*co*-acetoacetoxy methacrylate)] and Ag nanoparticles. A typical approach for the stabilization of Au nanoparticles in the hydrogel networks through thiol–ester bonds was developed by employing the *N*,*N*′-cystamine-bisacrylamide (CBA) crosslinker [29].

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doi:10.1016/j.polymer.2006.10.045

The current interest in biomedical applications is that a versatile system must show antibacterial activity towards germs on contact without releasing toxic biocides. Silver nanoparticles (AgNPs) are being considered as a non-toxic and environmentally friendly antibacterial material. However, they have poor binding affinity with surfaces. Therefore, more recent advances are aimed to discover promising paths to obtain Ag nanoparticles in gel networks. In this way, polymer–silver nanoparticle systems opened a new window for a different range of applications in the biomedical field and these approaches are most effective and safe. The AgNP/poly(*N*-vinyl-2-pyrrolidone) composite functioned as a bactericide is applied in complicated cases of infected burns and purulent wounds [30].

A promising antimicrobial coating of poly(2-hydroxylethyl acrylate)–poly(ethylenimine) networks (PHEA–PEI) loaded with Ag nanoparticles and modified with PEG was reported for biomedical or daily-life applications [21]. The combination of metal nanoparticles (silver) with smart polymeric architectures seems to be a promising route to the design of novel materials. Thus herein, we explored the feasibility of precisely producing silver nanoparticles in hydrogel networks and demonstrated their excellent antibacterial activity.

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAM), sodium acrylate (SA), ammonium persulfate (APS), *N*,*N*′-methylenebisacrylamide (MBA), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), sodium borohydride ($NaBH_4$) were obtained from Aldrich (USA). Silver nitrate ($AgNO_3$) was purchased from Junsei Chemical Co., Ltd (Japan). All the chemicals were used as such without any further purification. Double-distilled water was used for making solutions and polymerizations as well.

2.2. Methods

NIPAM (800 mg), SA (200 mg) and MBA (14 mM) were added in 8 mL of distilled water. To this homogeneous monomer solution, each 1 mL of APS (1 g/100 mL water) and TEMED (1 g/100 mL water) were added sequentially under continuous stirring. The free-radical crosslinking polymerization in a test tube was continued for 24 h at room temperature in order to get complete network formation. After breaking the tube, the gel rod was cut into several discs. Hydrogel discs were thoroughly washed with distilled water for three days to leach out any un-reacted chemicals. Similarly, by varying the MBA concentration (4.5, 6.00, and 8.5 mM) we obtained hydrogel with different network structures.

The preparation of Ag nanoparticles in the hydrogel networks has been performed in the following steps. Dry gel ($\sim$50 mg) was placed in 30 mL of water and allowed to swell completely. Then the swollen hydrogel was transferred to another beaker containing 30 mL of 5 mM silver nitrate solution to get silver ion loaded gels. After one day, Ag^+ loaded gels were placed in a 25 mL of 10 mM sodium borohydride solution. As soon as gel containing Ag salt contacted with $NaBH_4$ solution, the color of gel was immediately changed to brown. The obtained gel–Ag nanohybrids were characterized after grinding by using a ball mill. Silver nanoparticles were also prepared via the common technique of $AgNO_3$ reduction with $NaBH_4$ in the absence of hydrogel networks. *Escherichia coli* was used to test the biological potential of the Ag nanoparticles in the hydrogel networks. The antibacterial activity of the Ag nanoparticles was determined by standard methods. Petri plates were coated with the Ag nanoparticles in the hydrogel networks by evaporating a drop of solution (the test solutions were prepared in distilled water). Cells of the ubiquitous bacterium *E. coli* were then applied by aerosol spraying and cultivated under growth agar at 35 ± 2 °C for 24 h. The bacterium was cultured in Muller Hinton Broth (MHB) medium and used as inoculum for the study. *E. coli* cells (ATCC 25922, 10^7 cells/mL distilled water) were sprayed onto the substrate using a chromatography sprayer, dried for 2 min, and then the growth agar, Muller Hinton Agar (MHA), (0.7% in culture medium) was added. As expected, the number of grown colonies on the Ag nanoparticles in hydrogel networks was found to be almost nil when compared to the surrounding uncoated parts of the substrate.

2.3. Characterization

Well-dispersed hydrogel–Ag nanohybrid solutions (1 mg/1 mL water) were used for measuring the optical properties. UV–vis spectra were recorded over 300–700 nm range with 1 nm resolution using a Carry 1E UV–vis spectrophotometer (Varian 95011211). XRD analyses were performed with a Rikagu diffractometer (Cu radiation, $\lambda = 0.1546$ nm) running at 40 kV and 40 mA. Scanning electron microscope (SEM) images were taken with a Hitachi S-4700 (Japan) operating at an acceleration voltage of 15 kV. Glass transition temperature (T_g) for hydrogels was performed using Mettler Toledo Model DSC822e instrument. These experiments were performed for dry samples (5 mg) from 25 to 300 °C at a heating rate of 5 °C/min under 40 mL/min nitrogen flow. SEM specimens were prepared by placing 2–3 drops of gel–Ag nanohybrid solution on a silicon wafer and dried in air, followed by coating with Pd. Transmission electron microscopic (TEM) measurements were performed with a JEOL JEM-2000 operating at 200 kV. The sample was prepared by dispersing 2–3 drops of gel–Ag solution on 3 mm copper grid and removing excess solution using a filter paper and dried the grid for a day.

3. Results and discussion

Because of the outstanding applications of the gel–nanoparticle hybrids in various fields including biomedical applications, we were interested in developing a facile process to prepare a novel hydrogel–Ag hybrid. The concept of the present hydrogel network carrier approach is schematically presented in Fig. 1. This approach is useful to precisely produce Ag nanoparticles. Another main advantage of this

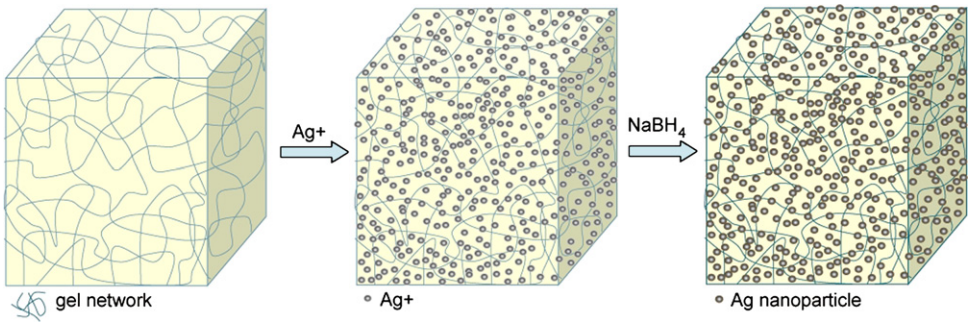

Fig. 1. Schematic representation for the Ag nanoparticles formation in the hydrogel network.

methodology is that it provides uniformly distributed Ag nanoparticles within the networks and the formed hydrogel–Ag networks can be effectively used for antibacterial applications. The hydrogel networks comprising PNIPAM and PSA chains were prepared as described in Section 2 and these networks were directly used as carriers for producing silver nanoparticles. The main reasons to select the poly(*N*-isopropylacrylamide-*co*-sodium acrylate) hydrogel as a nanogenerator are given here: (a) the poly(*N*-isopropylacrylamide) (PNIPAM) hydrogel is an excellent thermosensitive and biocompatible material that exhibits a sudden change with environment, but it has limited applications due to a fixed LCST [31,32]. Therefore, many researchers sought to overcome this limitation by adding acrylic acid or hydrophilic units into the NIPAM polymer backbone, which could shift the LCST to higher temperatures [33–36]. (b) Poly(sodium acrylate) or sodium acrylate can be effectively employed as a reducing agent or stabilizer for nanoparticles [37–39].

The completely swollen pre-weighed hydrogel discs were loaded with the Ag^+ salt and then reduced with $NaBH_4$ in the networks for obtaining the gel–Ag nanohybrids and finally the product was thoroughly purified by ultra filtration with distilled water. The present hydrogel networks can facilitate the narrow dispersion of silver nanoparticles and the network chains also assist in digestive ripening of such particles without aggregation. Interestingly, we produced silver nanoparticles with diameters of 2.67 ± 0.97 nm using our current method. The results obtained are superior to those reported previously. Until now, there is no simple and facile methodology that has been adopted for making concise nanoparticles using hydrogel networks. In our novel and facile system, the sodium acrylate repeat units present in PNIPAM hydrogel networks not only modify the hydrogel properties, but also allow to achieve a good stabilization of the nanoparticles, since the sodium acrylate or poly(sodium acrylate) chains efficiently act as stabilizing agents for the AgNPs [37–39]. It is worth to note that the as-prepared Ag nanoparticles in the hydrogel networks are highly stable and did not show any signs of aggregation, even after storage for six months.

The silver nanoparticle formation within the hydrogel networks can be seen from the bulk gel disc, which became brown after the reduction reaction (Fig. 2a). It is hypothesized that the silver nanoparticles are immobilized throughout the hydrogel networks due to a strong localization of the Ag^+ ions within

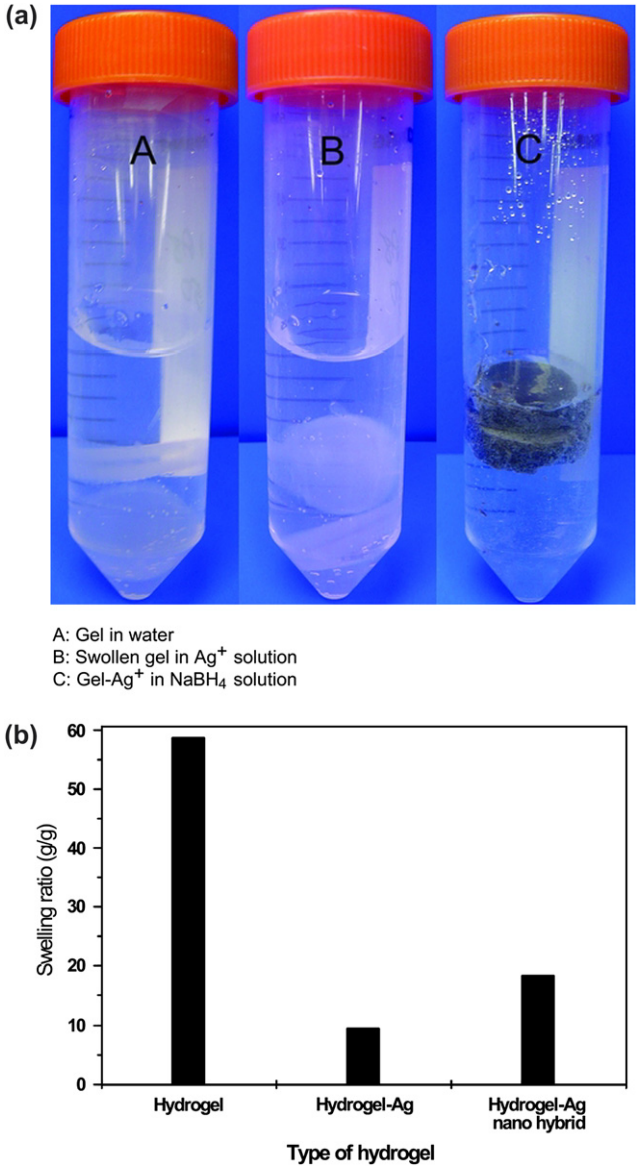

Fig. 2. (a) Photographs of the Ag nanoparticle formation in the hydrogels and (b) hydrogel swelling property.

the network. This is caused by the complexation of the Ag^+ ions by either the nitrogen atoms of PNIPAM and or the oxygen atoms (COO) of PSA. This is further confirmed from Fig. 2b, as a large shrinkage of the hydrogel after the addition

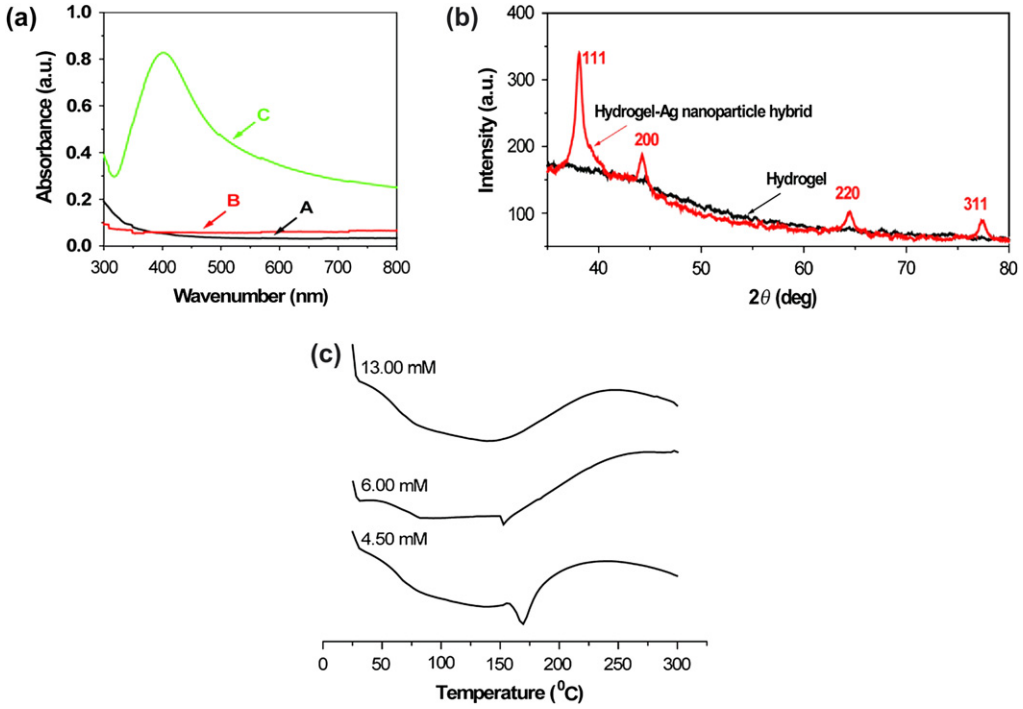

Fig. 3. (a) UV spectra of (A) hydrogel, (B) $AgNO_3$ and (C) hydrogel−Ag nanoparticle hybrids, (b) XRD patterns of hydrogel and hydrogel−Ag nanoparticle hybrids, and (c) DSC thermograms of hydrogels crosslinked with different amounts of MBA.

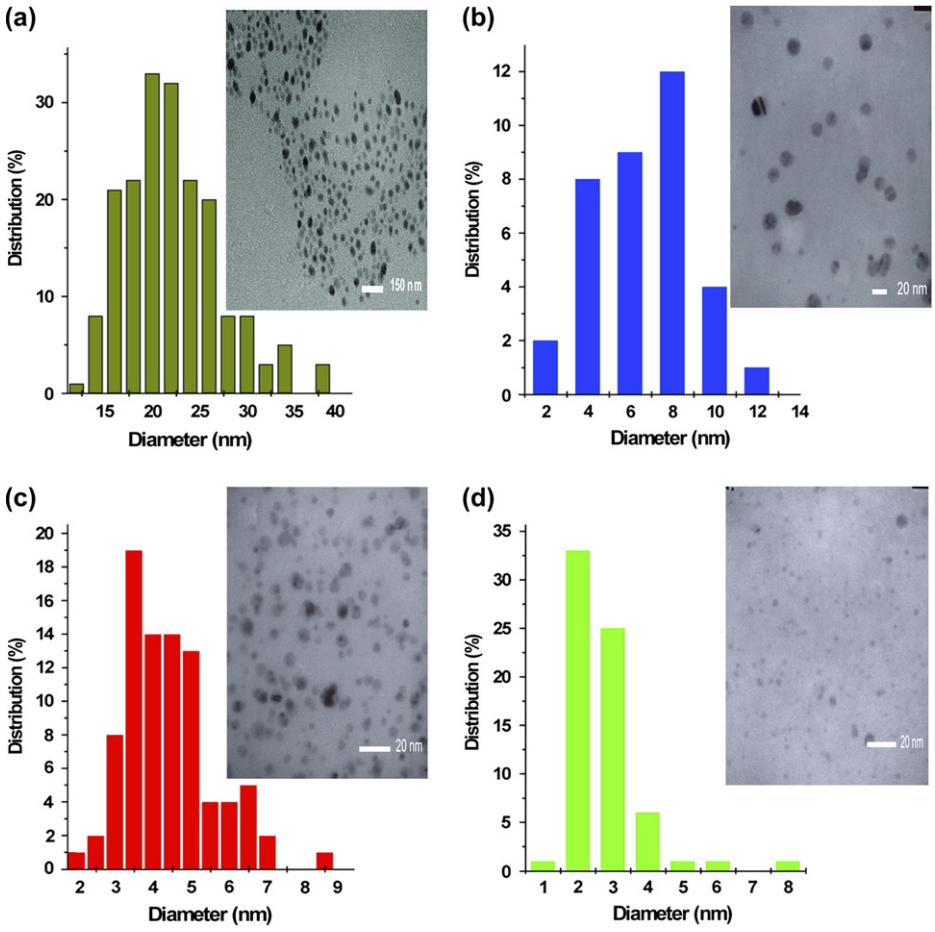

Fig. 4. (a–d) TEM and histograms of 21.11 ± 4.60, 6.63 ± 2.37, 4.36 ± 1.18, and 2.67 ± 0.97 nm Ag nanoparticles in 4.50, 6.00, 8.50, and 13.00 mM MBA-crosslinked hydrogels, respectively.

of $AgNO_3$. However, at the same time, the network system showed an improved swelling pattern after silver nanoparticles formation due to the surface charge of the nanoparticles (different size of the AgNPs) that perturb and cause an expansion in the networks of the gel. Therefore, the order of the swelling capacity of the gel is in the following order: pure hydrogel > hydrogel–Ag hybrid > hydrogel–Ag salt hybrid.

In order to inspect the formation of nanoparticles prepared using the contemporary approach, we have carried out UV–vis absorption studies and the corresponding results are shown in Fig. 3a. It is very clear that an absorption peak at around 404 nm is noticed for the AgNPs due to the surface plasmon resonance effect originating from the quantum size of silver nanoparticles [19,40]. A well-defined surface plasmon resonance at around 400 nm was observed for the 2.5 nm silver nanoparticles [41,42], whereas the pure hydrogel and $AgNO_3$ showed no absorbance in this UV–vis region (Fig. 3a).

Fig. 3b shows the XRD pattern of the hydrogel and the AgNPs in the hydrogel networks. The diffraction peaks for the Ag–hydrogel nanohybrid found at the 2θ value of 38.1°, 44.26°, 64.50°, and 77.42° are attributed due to (111), (200), (220), and (311) planes of the fcc silver, respectively, [43,44]. The pure hydrogel did not show any peaks in the XRD pattern, thus indicating no crystalline behavior.

The size and morphology of the nanoparticles could be controlled by varying the crosslinking density, functionalization, and modifying the networks of the hydrogels. To control the size of nanoparticles in the network of the hydrogel we prepared different crosslinking networks by employing various amounts of MBA crosslinker, i.e., 4.50–13.00 mM. The crosslinking density variation was confirmed through the glass transition temperature measurements of the hydrogels. The DSC curves of these hydrogels are presented in Fig. 3c. Hydrogel with a low crosslinking degree showed two glass transition temperatures at around 150 and 185 °C. The first T_g is may be due to poly(*N*-isopropylacrylamide) and the second one is due to poly(sodium acrylate). It was found that the glass transition temperature for different chain configurations of poly(*N*-isopropylacrylamide) (non-crosslinked) was between 136 and 142 °C [45]. In this study, it was observed that there was increment in the glass transition temperature of the NIPAM polymeric chains (first T_g), which could be due to crosslinked polymeric chains. Further, the second sharp glass transition temperature completely disappeared for the hydrogel prepared with higher crosslinker concentrations. This is obvious because an increase in the crosslinker concentration results in higher dense networks that leads to higher T_g values. It is important to note that the silver nanoparticles obtained in the different crosslinked networks using the current methodology showed highly dispersed spherical nanoparticles, but were found to be varied in their size. Fig. 4 explains that the MBA concentration in the preparation of hydrogel networks determines the size of silver nanoparticles. This strictly indicates that highly denser hydrogel networks (with a high MBA concentration) are responsible for obtaining lower sized silver nanoparticles (2.67 ± 0.97 nm), since they favor to establish more intra–inter molecular attractions between gel networks owing to less free space in the hydrogel networks (Fig. 4d). The denser networks would help to stabilize as well as regulate the size of nanoparticles. On the other hand, lower MBA-crosslinked gel networks provide enough free space to grow greater nano-sized particles. As the crosslinker concentration decreased from 13.00 to 4.50 mM, the nanoparticle size produced in the networks was increased from 2.63 to 21.11 nm.

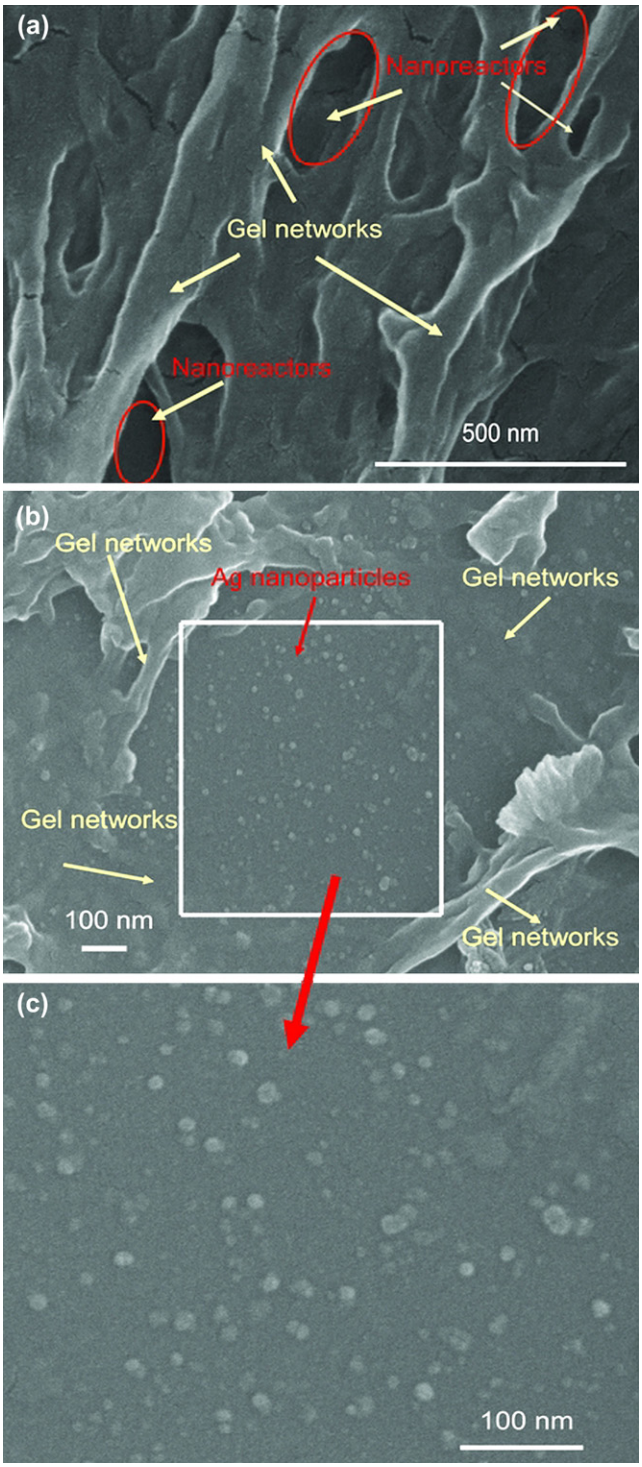

Fig. 5. (a) SEM of pure hydrogel and (b, c) Ag nanoparticle grown in 4.50 mM MBA-crosslinked hydrogel network nanoreactor.

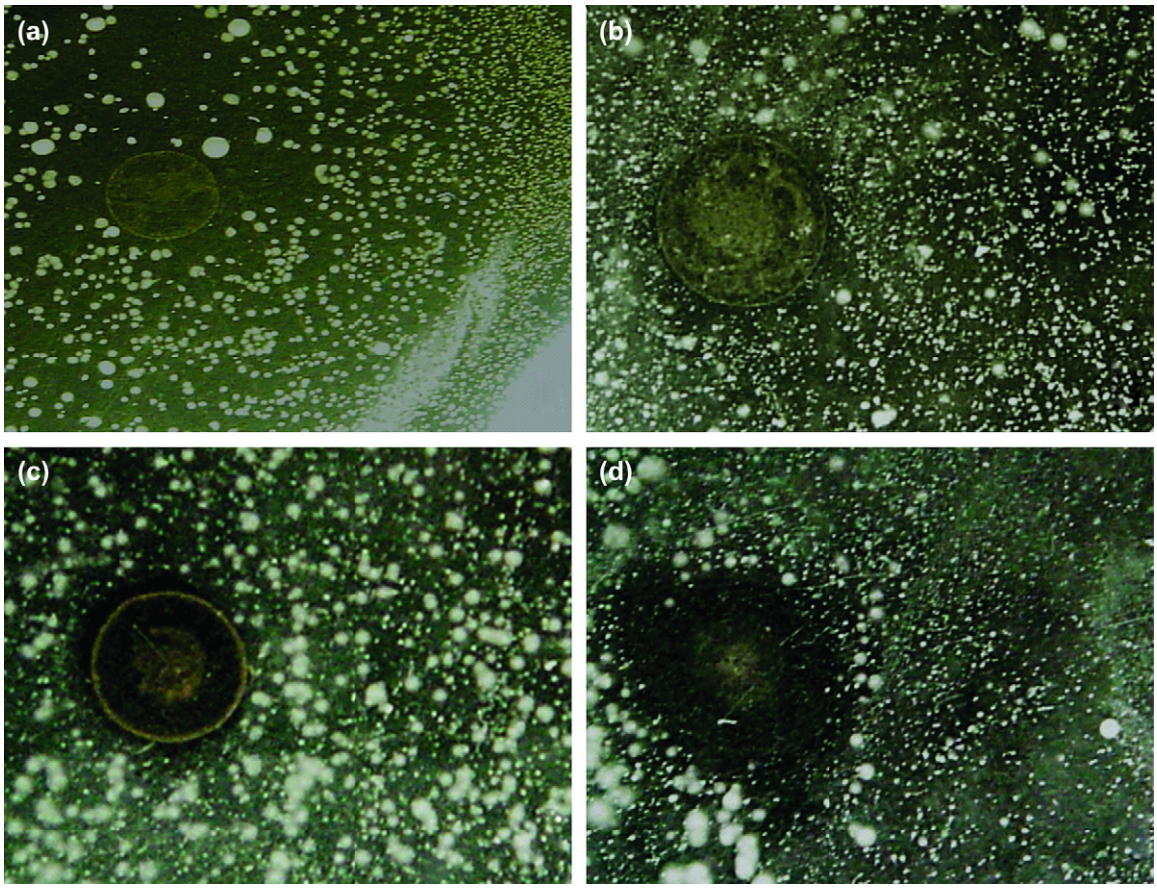

Fig. 6. Antibacterial results with the hydrogel–Ag nanoparticle hybrid.

Further, the nanocarrier or nanoreactor behavior of the hydrogel networks could be seen from Fig. 5. It eventually shows that the growth of the nanoparticles between the networks of the hydrogel and nanoparticles is uniformly distributed between the chains, whereas the blank hydrogel (without nanoparticles) showed clear networks throughout the gel structure. Moreover, this methodology is a general model for making nanoparticles within the networks. In the case of silver nanoparticles embedded in the networks they are fully accessible only below the LCST. The incorporation of sodium acrylate units into the PNIPAM hydrogel networks, definitely improves the LCST and favorable networks for making gel–nanoparticle hybrids even at higher temperatures, and thereby extends their applications not only in biomedical but also in catalysis.

By employing the present novel hydrogel–silver nanoparticle hybrid systems, we studied the antibacterial activity test towards *E. coli*. The influence of the gel hybrids containing the silver nanoparticles of different size, i.e., 2.67 ± 0.97 nm, 6.63 ± 2.37 nm, and 21.11 ± 4.60 nm, were examined and compared with silver nanoparticles ($\sim$220 nm) without any stabilization. The results suggest that the silver nanoparticles of $\sim$2.67 nm protected by hydrogel polymer chains (Fig. 6d) showed an excellent antibacterial activity compared to the larger sized silver nanoparticles in the hybrid networks (Fig. 6b and c). The reason might be due to the fact that the lower size nanoparticles in the polymer networks could come out easily and could interact with *E. coli*, whereas the larger sized silver nanoparticles might have a reduced diffusion tendency. We also observed that, even if the AgNP can leave the hydrogel network, they do not show a high antibacterial activity, if the size of the silver nanoparticles is large. This phenomenon is further confirmed with the 220 nm silver nanoparticles (metal reduction method in the absence of the networks) in Fig. 6a. From these results, we conclude that the hydrogel-stabilized silver nanoparticles (2.67 nm) would be the better choice for antibacterial activity.

4. Conclusions

In summary, we have demonstrated a facile strategy to produce well-defined and precise silver nanoparticles ($\sim$3 nm) within the hydrogel networks and explored its application as antibacterial material. This methodology could also applicable for producing other metal nanoparticles.

Acknowledgments

We thank the Korean Research Foundation (KRF) and the Ministry of Education (PostBK21 project) for the financial support.

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