

High antimicrobial activity of new eco-friendly and biocompatible nanocomposite based on the AgCl-decorated poly(sodium acrylate) cryogel

Elham Yadegari^{*1}, Sajedeh Abedi², Amirhosein Khademi³

Received: 2024-10-29
Revised: 2024-11-01
Accepted: 2024-11-01
DOI: [10.61186/CNJ.2.2.296](https://doi.org/10.61186/CNJ.2.2.296)

¹Isfahan University of Medical Sciences faculty of pharmacy Hezar Jerib Blvd, Isfahan, Iran

²Department of chemistry, Payame noor University, Arak, Iran

³Faculty of Medical Science, Islamic Azad University of Khomein, Khomein, Iran

Abstract

This study addresses the preparation of antibacterial agent-decorated cryogels, AgCl nanoparticles-decorated poly(sodium acrylate) (AgCl/PSA) cryogel at different AgCl Wt.%, and their antibacterial application investigation. Cryogels are formed by conducting a polymerization reaction in a semi-frozen system where the ice crystals (for aqueous systems) act as the porogens, resulting in a highly interconnected porous network. The prepared samples were characterized with XRD, AFM, and SEM. AFM images show that the AgCl nanoparticles concentration decorated on the surface of PSA cryogel influenced the surface topography of cryogel nanocomposites. *Escherichia coli* and *Bacillus subtilis* were selected as exemplary gram-negative and gram-positive bacteria for antibacterial testing. The prepared cryogel nanocomposite samples showed a 4.5-7.0 log reduction of viable bacteria in the antibacterial test. The PSA/AgCl-10 cryogel as the best sample was also highly reusable over six cycles of antibacterial test operation. Due to their simple operation, ease of deployment, and high antibacterial performance, the synthesized AgCl-decorated cryogels offer great promise for different applications.

Keywords: Antimicrobial resistance, AgCl nanoparticles, Poly(sodium acrylate), Cryogel, Nanocomposite

1. Introduction

Antimicrobial resistance has been seriously threatening public health and the COVID-19 pandemic has worsened this issue. In order to global economic development and public health, it is obvious that there is an urgent need to discover new antimicrobial agents based on the nanotechnology [1]. Especially those that have high antibacterial activity against Gram-negative bacteria due to the unique structure of the cell envelope. One of the greatest threats to public health is microbiological contamination of drinking water sources. Approximately 1.8 million people die annually from diarrheal diseases. Most of these deaths are children. Improving microbiological water quality at the point of use could reduce the risk of diarrheal diseases [2,3]. However, the effectiveness and/or formation of harmful disinfection by-products of conventional disinfection methods is limited. In addition, the emergence of micro-organisms that are resistant to several antimicrobial agents calls for the development of improved disinfection methods that minimize the formation of disinfection by-products [4]. Due to their high interfacial reactivity and unique physicochemical properties, nanoscale materials have attracted increasing interest as alternative disinfectants. Especially silver (Ag) nanoparticles (Ag NPs) have been shown to have outstanding antimicrobial activity [5]. However, the practical use of free Ag NPs is still limited due to dispersion and dissolution problems. These problems can lead to loss of efficacy and potential (eco)toxicological effects. These issues can be addressed by stabilizing Ag NPs in various inorganic/organic carriers [6-8]. The application of AgNPs-functionalized ceramic filters, hydrogels, ion-exchange materials, papers, polyurethane foam and polymer beads as bioactive components in flow or column systems for point of use water disinfection has been tested [9].

These studies have demonstrated the efficacy of Ag nanocomposites in terms of bacterial deactivation. However, little attention has been paid to elucidating their bactericidal mechanisms. In addition, their applications may be limited by either: (i) a relatively low output of disinfected water or (ii) a significant release of Ag into the treated water, compromising the potability of the water and the

reusability of the nanocomposites. In addition, systems operated in the column mode may have some operating limitations. The development of antibacterial agents with a combination of nanoparticles and a matrix have been a challenge [10-13]. Fortunately, via the structural modification of nanoparticles and effective dispersion of nanoparticles this fact has been approved for several application fields and also marketing. Consequently, silver chloride (AgCl) as a sustainable silver ion source is a potential candidate for treating infections. In addition, AgCl in the form of nanoparticles may be more toxic to bacteria than its bulk counterpart. This is because nanoparticles of small size can pass through cell membranes, and the accumulation of intracellular nanoparticles can lead to cell dysfunction [14]. Cryogels are formed by conducting a polymerization reaction in a semi-frozen system in which the ice crystals (for aqueous systems) act as the porogens, resulting in a highly interconnected porous polymer network or matrix [15]. This is in contrast to hydrogels which are formed at higher temperatures, such as 5° C. or above, not in a semi-frozen aqueous system. Briefly, a hydrogel refers to a polymeric soft matter that is or can be swollen with water. Cryogels belong to a special class of gel-like polymers that are prepared below freezing point of the solvent (for example water). Such special process renders the cryogels their characteristic macroporous structures and other unique attributes including mechanical properties and water absorption/releasing behaviour desirable for the present application. For example, using poly(sodium acrylate) (PSA) as the polymeric matrix, PSA hydrogels and PSA/nanoparticles hydrogels were prepared using the same reagents and formulation as the PSA cryogels except that the gelation was conducted at room temperature (freezing is required for cryogels, hence the word “cryo”) [16].

Here we reported the preparation of antibacterial agent-decorated cryogels, AgCl nanoparticles-decorated cryogel at different AgCl Wt.% in cryogel matrix, and their antibacterial application investigation. Cryogels are formed by conducting a polymerization reaction in a semi-frozen system in which the ice crystals (for aqueous systems) act as the porogens, resulting in a highly interconnected porous network. The design of present AgCl-decorated cryogels combines the advantages of high porosity, excellent mechanical and water absorption properties of cryogels, and uniform dispersion of fine AgCl nanoparticles on the cryogel pore surface for rapid disinfection with minimal Ag release. Present AgCl nanoparticles-decorated cryogels are lightweight and permit easy recovery of the absorbed (i.e. disinfected) water via the application of minimal pressure, e.g. by manual hand compression. *Escherichia coli* (E. coli, ATCC® 25922™) and *Bacillus subtilis* (B. subtilis, ATCC® 6633™) were selected as exemplary gram-negative and -positive bacteria for antibacterial testing. Due to their simple operation, ease of deployment and high antibacterial performance, the synthesized AgCl-decorated cryogels offer great promise for different application and especially can be used as a potable wastewater treatment filter cartridge to obtain clean water especially in emergencies where there is limited access to the infrastructure.

2. Experimental

2.1. Materials

N,N,N',N'-tetramethylethylenediamine (TEMED, ≥99) as accelerator of polymerization, sodium acrylate (SA, ≥99%), N,N'-methylenebis(acrylamide) (MBA, 99%) as cross-linker were purchased from Sigma-Aldrich. Ammonium persulfate (APS, 98%) as hydrophilic initiator, PVA (polyvinyl alcohol) surfactant, AgNO₃ and NaCl were purchased from Merck.

2.2. P(sodium acrylate) (PSA) cryogels synthesis

The design principles and synthesis of PSA cryogels have been described previously [17]. In brief, a desired amount of ammonium persulfate initiator and N,N,N',N' tetramethylethylenediamine were added to a reaction mixture containing sodium acrylate and N,N'-methylenebis(acrylamide) as cross-linker agent. The obtained mixture was degassed and cooled in an ice bath. In this recipe, final ammonium persulfate and N,N,N',N'-tetramethylethylenediamine concentrations were 1.75 mM and 0.125% (v/v), respectively, and the monomer concentration (sodium acrylate + N,N'-methylenebis(acrylamide)) used was 8%. The mol ratio of crosslinker to sodium acrylate was 0.05. The resulting reaction mixture was transferred to several poly(propylene) syringes (5 mL), which were then placed in a bath fluid (-20°C, 1:1 mixture of ethylene glycol/double distilled water (19.02 MΩ·cm at 25°C)) and incubated in an ultra-low temperature freezer (Portable/Benchtop Ultra-Low Temp Freezer (-86°C), 120 V). After 24 h, the PSA cryogels were washed thoroughly in ultra-pure water and dehydrated in n-butanol. Then dried in a lyophilizer (Pharmaceutical Laboratory Lyophilizer Freeze Dryer Mini Vacuum Freeze Dry Machine CE Certified) before being broken into smaller cylindrical disc samples.

2.3. Preparation of AgCl nanoparticles-impregnated PSA cryogels

For the preparation of PSA/AgCl cryogels nanocomposites, the inter matrix synthesis (IMS) method was used. Typically, AgCl nanoparticles were synthesized by the chemical reaction between silver ions from AgNO₃ and chloride ions from NaCl in the presence of the stabilizer PVA according to the following procedure: 1.634 g PVA and 0.122 g of AgNO₃ were dissolved in 210 ml of distilled water. To this solution, 14.4 ml of 0.1 M NaCl aqueous solution (1.44 mmol) was added at a constant rate over 30 min with vigorous stirring [18]. The reaction mixture was stirred for a further 2 h at room temperature and then different amount of dried PSA cryogel was allowed to swell into the above solution to adjust the Wt.% of AgCl nanoparticles on the surface of PSA cryogel and the preparation a series of AgCl decorated PSA cryogel nanocomposites. All suspensions were shaken at 250 rpm on an orbital shaker for 24 h. The resulting swollen cryogel samples were washed several times with water to remove the unbound Ag⁺ ions on the surface of cryogel matrix. The resulting nanocomposites were thoroughly washed by immersion in water followed by vacuum filtration for 3 times. After three repetitions of the washing step, the nanocomposites were dried using the same procedure as for the PSA cryogel. The prepared sample based on the used weight percentage of AgCl nanoparticles on the surface of PSA including 5 Wt.%, 10 Wt.%, and 15 Wt.%, were marked as AgCl/PSA-5, AgCl/PSA-10 and AgCl/PSA-15. In this study as control sample for comparison investigation, pure PSA cryogel without AgCl nanoparticles, and unsupported AgCl nanoparticles were prepared to confirm the role of PSA for antibacterial activity enhancement.

2.4. Characterization

X-ray diffraction (XRD) is a powerful nondestructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects. In this study, an XRD (Philips, X'Pert- MPD PRO-PW3040/60) was used. The FESEM as the most common technique to use in the characterization of surface topography of materials and morphological differences on the surface was performed by (TESCAN MIRA LMU). AFM was used to investigate the surface topography of the prepared samples compared to the PSA cryogel with AFM (BRISK).

2.5. Antibacterial activity testing

Escherichia coli (E. coli, ATCC® 25922™) and Bacillus subtilis (B. subtilis, ATCC® 6633™) were selected as exemplary gram-negative and -positive bacteria for antibacterial testing. E. coli and B. subtilis were cultured in tryptic soy broth and nutrient broth at 37°C and 30°C, respectively, and harvested at mid-exponential growth. The harvested cells were washed by centrifugation and then resuspended in phosphate buffered saline (PBS, 0.01 M, pH = 7.45) [19]. A 0.02 g of all AgCl/PSA samples was added to a 10 mL bacterial suspension with a cell density of 10⁸ colony forming units per mL (cfu mL⁻¹). The cryogel was manually shaken during swelling in the bacterial suspension. The swollen cryogels were quickly removed and squeezed to obtain the treated water after 15 s of swelling in the bacterial suspension. Control experiments were performed without the addition of cryogel to the bacterial suspension. After appropriate dilution in PBS, 0.1 mL of the control, treated water and bulk water were plated on tryptic soy agar or nutrient agar and incubated for 24 h to enumerate the number of viable bacteria. At least 6 replicate experiments were performed. The kinetics of bactericidal activity was studied by quenching of the disinfection reaction with universal quencher (0.1% peptone; 0.1% Na₂S₂O₃; 0.5% Tween 80, 0.07% lecithin). The bacterial suspension was diluted 10-fold in Universal Quenching Agent (UQA) to quench the reaction.

3. Results and discussion

3.1. AFM

The AFM analysis with 3-D images are shown in Fig. 1. As can be seen from Fig. , it is obvious that the pure PSA cryogel has a uniform surface with homogeneous porosity compare to the prepared AgCl/PSA cryogel samples. Fig. 1, shows AgCl nanoparticles concentration decorated on the surface of PSA cryogel influenced the surface topography of AgCl/PSA cryogel nanocomposite with surface roughness measured to be 29 ± 6 nm, 38.16 ± 2 nm and 68 ± 1 nm for (b) AgCl/PSA-5, (c) AgCl/PSA-10 and (d) AgCl/PSA-15, respectively. Moreover, AgCl nanoparticles loading into the PSA cryogels further improved their mechanical properties since it resulted in stronger and more rigid nanocomposites compared to the pure PSA.

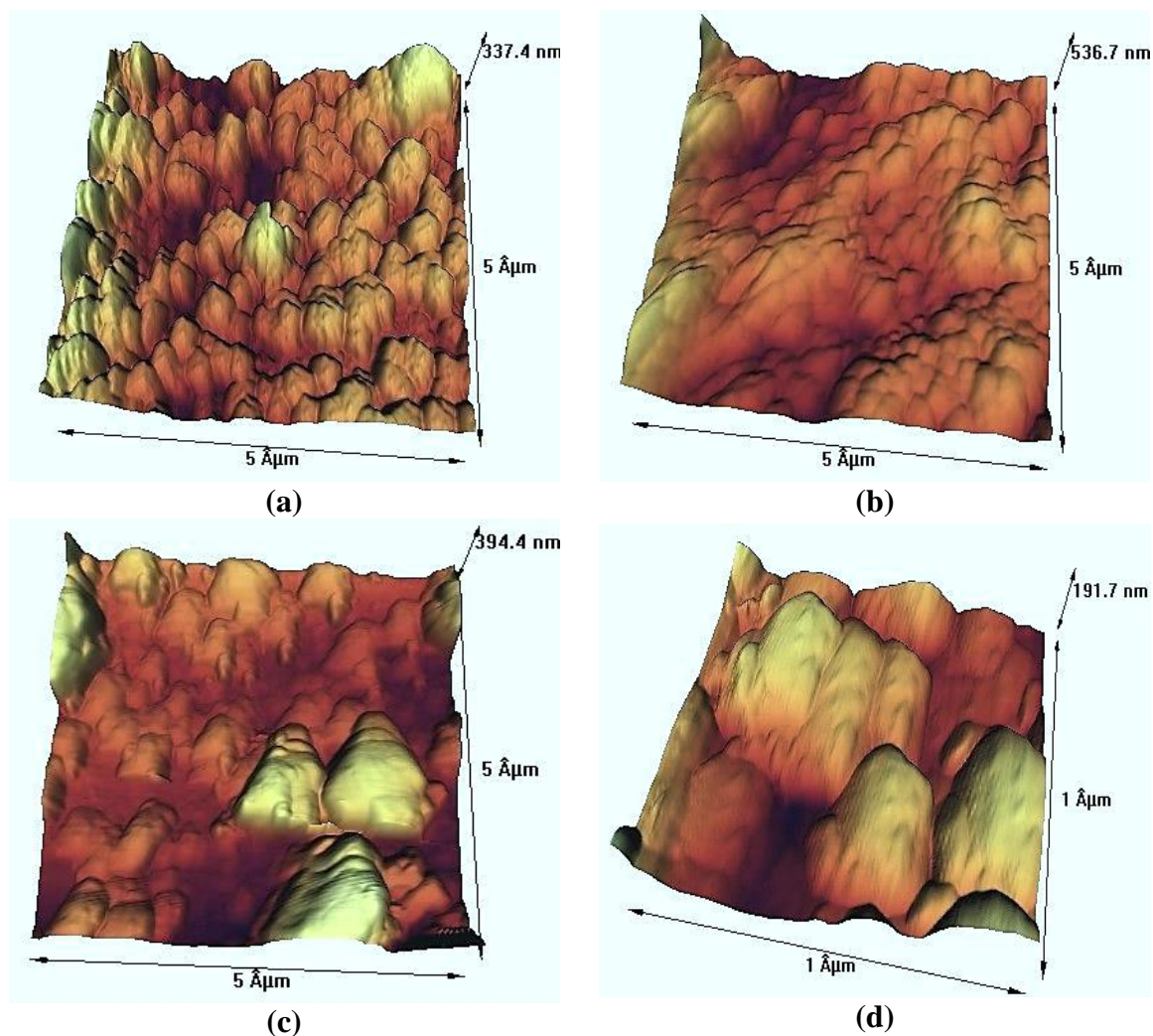


Fig. 1. AFM images of the lyophilized (a) PSA, (b) AgCl/PSA-5, (c) AgCl/PSA-10 and (d) AgCl/PSA-15 cryogel nanocomposites

3.2. Crystalline nature study

The XRD pattern was used to investigate the crystalline nature of the obtained AgCl/PSA cryogel nanocomposite as a typical sample. The diffraction sharp peaks observed in the 2θ range from 20° to 80° in the XRD spectrum of the lyophilized AgCl/PSA cryogel powder were 27.97° ; 32.32° ; 46.24° ; 54.79° ; 57.55° ; 67.63° ; 74.50° ; 76.54° (Fig. 2). They are assigned to the (111), (200), (220), (311), (222), (400), (331) and (420) planes, respectively, of the face-centered cubic structure of AgCl crystal in PSA cryogel matrix [18].

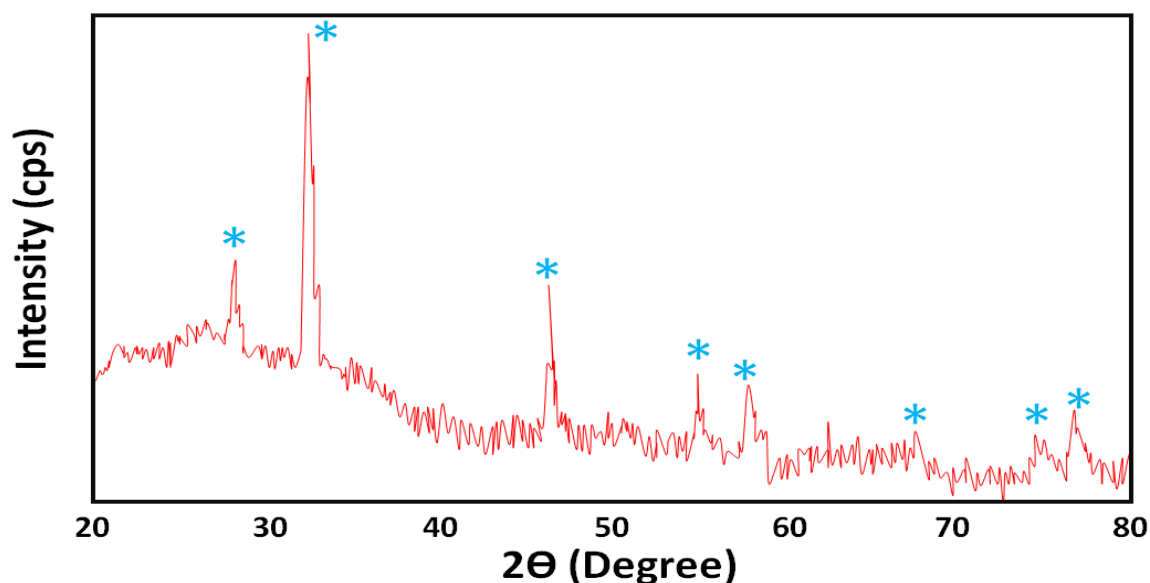


Fig. 2. XRD pattern to investigate the crystalline nature of the lyophilized AgCl/PSA-10 cryogel powder from $2\theta = 20^\circ - 80^\circ$

3.3. FESEM of lyophilized AgCl/PSA cryogel

Fig. 3 shows the FESEM image of the AgCl/PSA-10 cryogel sample. This shows that the pore size and interconnectivity of the cryogel were relatively unaffected after decorating with AgCl nanoparticles. This is due to the large size difference between cryogel pores (1-100 μm) and AgCl nanoparticles (mostly $<20\text{ nm}$). The AgCl nanoparticles with 10 Wt.% loading was very well dispersed in the cryogel network, with bright spots appearing on the surface of the cryogel pore walls. The good dispersion can be attributed to the electrostatic stabilization of the AgCl nanoparticles in the PSA cryogel. Furthermore, the highly cross-linked polymer network of PSA provided random confinement of the AgCl nanoparticle growth in the free volumes between the networks. The particles were mostly $<20\text{ nm}$ in size. Highly interconnected porous network of PSA cryogel combined with the good dispersion of fine AgCl nanoparticles is anticipated to increase the probability of collisional contact with bacterial cells that would lead to high disinfection efficacies and high antibacterial activity.

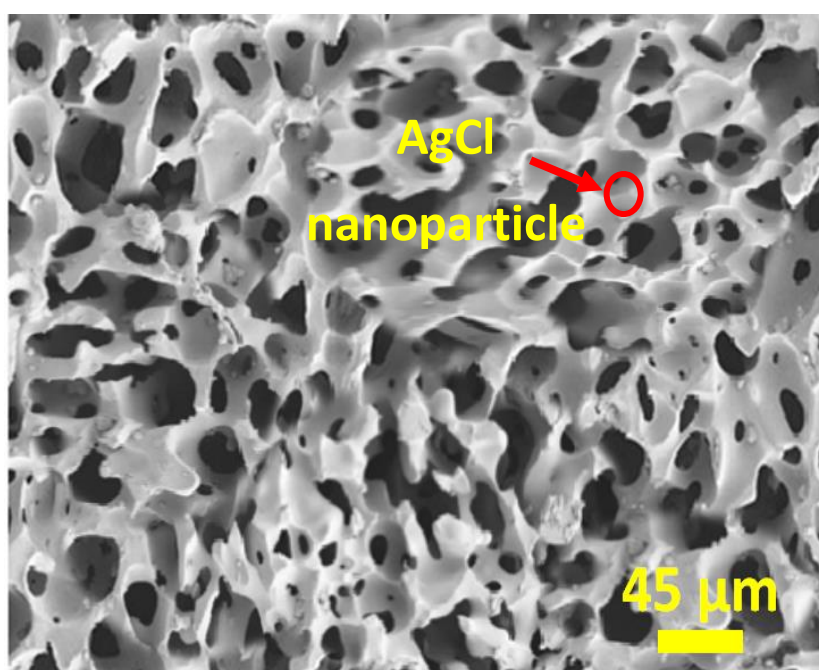


Fig. 3. FESEM of the lyophilized AgCl/PSA-10 cryogel powder

3.4. Antibacterial test results

The PSA/Ag cryogels synthesized in this study showed excellent antibacterial activity against both *E. coli* and *B. subtilis* compared to the control samples pure PSA and AgCl nanoparticles. The prepared cryogel nanocomposite samples showed a 4.5-7.0 log reduction of viable bacteria in the antibacterial test (Fig. 4). It should be noted that even the AgCl/PSA-5 cryogel having the lowest Ag content could deactivate more than 4logs of viable bacteria (Fig. 4). As can be seen from Fig. 4, the antibacterial performance for *B. subtilis* in high content of AgCl is more and *B. subtilis* was deactivated to a greater extent than *E. coli*. Unmodified PSA cryogels without AgCl nanoparticles have low antibacterial activity and this low value to remove some bacteria could possibly due to (i) bacterial exclusion by smaller pores, (ii) bacterial entrapment in blind pores, and/or (iii) deposition of bacterial cells on the interior surface of cryogel during compression [20]. However, the extent of reduction was marginal when compared with that of the PSA/AgCl cryogels.

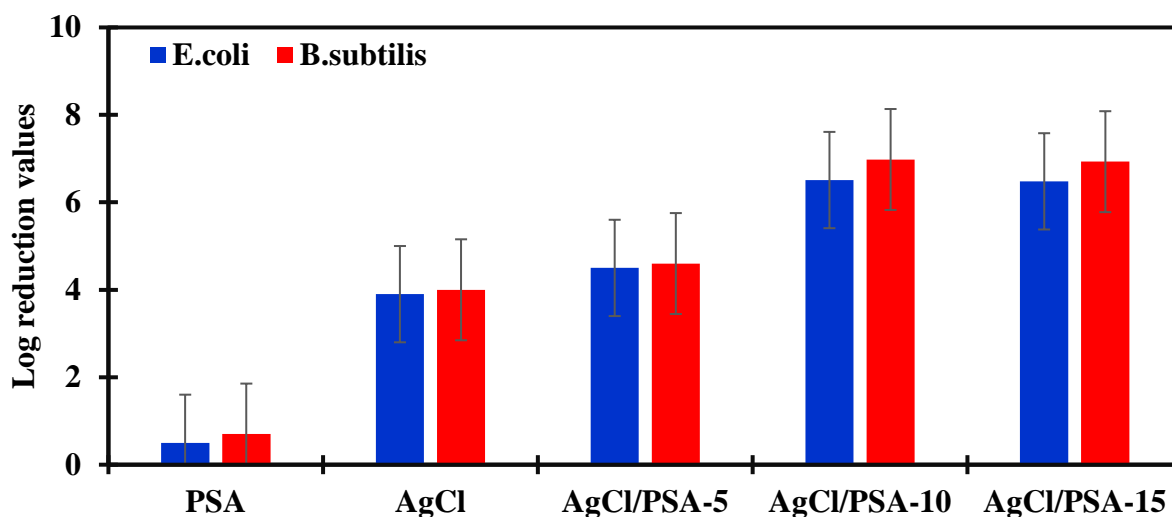


Fig. 4. Log reduction values VS. all samples to compare antibacterial activity

AgCl/PSA-10 was selected for bactericidal reusability study due its excellent disinfection efficacy. The PSA/AgCl-10 cryogel was also highly reusable as indicated by the relatively consistent log reduction values over six cycles of antibacterial test operation (Fig. 5). As shown in Fig. 5, the log reduction values were not statistically different between the 6 operational cycles. The good reusability of PSA/AgCl cryogel may be attributed to the high stability of AgCl nanoparticles incorporated in PSA cryogel matrices.

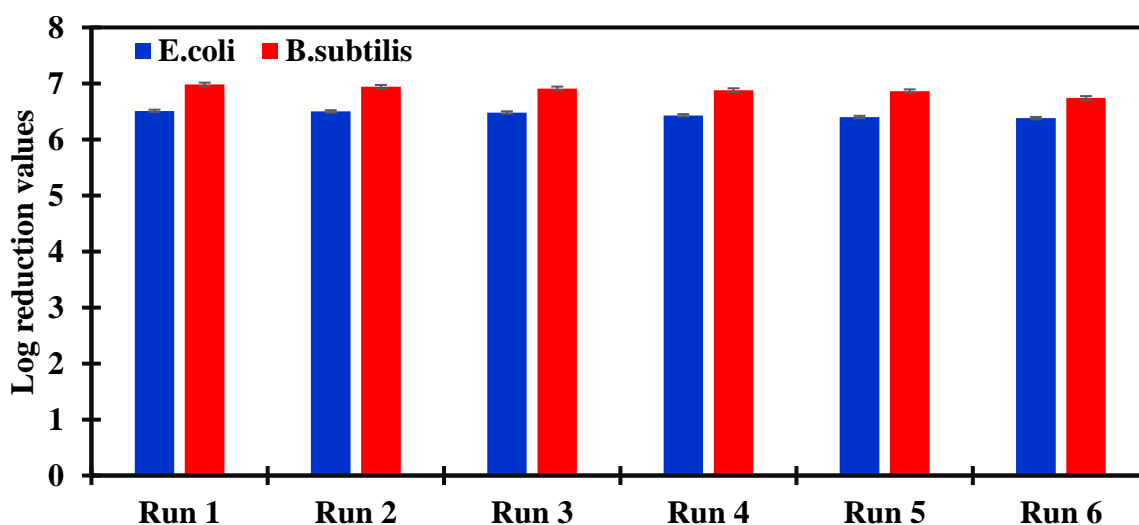


Fig. 5. The reusability study of AgCl/PSA-10 cryogel sample

The mechanisms by which free AgCl nanoparticles exert toxicity have been studied; however, there is no general consensus as to whether the toxicity of the AgCl nanoparticles was due to release of Ag⁺ ions or to intrinsic properties specific to the particle (primarily Ag⁰). Ag⁺ ions are toxic to bacteria due to various mechanisms including binding to thiols in proteins and disrupting the bacterial respiratory chain, thereby generating reactive oxygen species (ROS) that can lead to oxidative stress and cell damage. On the other hand, the toxicity effects of AgNPs have been suggested to arise from: (i) physical processes that involve disruption of the cell membrane and/or penetration of AgCl nanoparticles into the cell, (ii) particle surface reactions that generate ROS, which catalyzes the oxidation of cellular contents, and/or (iii) direct interaction with enzyme sites that changes the conformation resulting in impaired metabolism. The bactericidal mechanism of bulk materials functionalized with AgCl nanoparticles is rarely discussed in the literature. Present work hypothesizes that the biocidal action of AgCl nanoparticles/PSA cryogels is dominated by surface-controlled mechanisms that are dependent on direct contact of the interface of the AgCl/PSA cryogels with the bacterial cells [20]. This indicates that the bacterial cells need to come into close contact with AgCl/PSA cryogels

Therefore, it is believed that the AgCl/PSA cryogels prepared in this study may offer a simple approach for drinking-water disinfection as a wastewater cartridge filter in disaster-relief applications. In addition, the AgCl/PSA cryogels prepared in this study are lightweight and highly portable allowing it to be easily deployed for emergency response.

4. Conclusion

In this study, the antibacterial agent-decorated cryogels, AgCl nanoparticles-decorated poly(sodium acrylate) (AgCl/PSA) cryogel at different AgCl Wt.%, and their antibacterial application was considered. The prepared samples were characterized with XRD, AFM, and SEM. *Escherichia coli* (*E. coli*, ATCC® 25922™) and *Bacillus subtilis* (*B. subtilis*, ATCC® 6633™) were selected as exemplary gram-negative and -positive bacteria for antibacterial testing. The prepared cryogel nanocomposite samples showed a 4.5-7.0 log reduction of viable bacteria in the antibacterial test. The PSA/AgCl-10 cryogel as the best sample was also highly reusable over six cycles of antibacterial test operation. Due to their simple operation, ease of deployment, and high antibacterial performance, the synthesized AgCl-decorated cryogels offer great promise for different applications.

Conflicts of Interest

The author declares no conflict of interest.

Author information

*Corresponding Author: Elham Yadegari

E-mail address: elham.yadegari@gmail.com

References

- [1] E. Ponzo, S. De Gaetano, A. Midiri, G. Mancuso, P. Giovanna, D. Giuliana, S. Zummo, C. Biondo, The antimicrobial resistance pandemic is here: implementation challenges and the need for the one health approach. *Hygiene*, 4(3) (2024) 297-316. <https://doi.org/10.3390/hygiene4030024>
- [2] Y. Fu, Q. Dou, K. Smalla, Y. Wang, T.A. Johnson, K.K. Brandt, Z. Mei, M. Liao, S.A. Hashsham, A. Schaffer, Gut microbiota research nexus: One Health relationship between human, animal, and environmental resistomes. *MLife*, 2 (2023) 350–364. <https://doi.org/10.1002/mlf2.12101>
- [3] F. Prestinaci, P. Pezzotti, A. Pantosti, Antimicrobial resistance: A global multifaceted phenomenon. *Pathog. Glob. Health*, 109 (2015) 309–318. <https://doi.org/10.1179/2047773215Y.0000000030>
- [4] B. Chala, F. Hamde, Emerging and re-emerging vector-borne infectious diseases and the challenges for control: a review. *Front. Public Health*, 9 (2021) 715759. <https://doi.org/10.3389/fpubh.2021.715759>
- [5] T. Jaswal, J.A. Gupta, A review on the toxicity of silver nanoparticles on human health. *Mater. Today Proc.* 81 (2023) 859–863. <https://doi.org/10.1016/j.matpr.2021.04.266>
- [6] A. Salabat, F. Mirhoseini, M. Mahdieh, H. Saydi, A novel nanotube-shaped polypyrrole-Pd composite prepared using reverse microemulsion polymerization and its evaluation as an antibacterial agent, *New J. Chem.* 39 (5) (2015) 4109–4114. <https://doi.org/10.1039/c5nj00175g>

- [7] F. Kamali, K. Faghihi, F. Mirhoseini, F. High antibacterial activity of new eco-friendly and biocompatible polyurethane nanocomposites based on $\text{Fe}_3\text{O}_4/\text{Ag}$ and starch moieties. *Polym. Eng. Sci.*, 62(5) (2022) U1444-1462. <https://doi.org/10.1002/pen.25934>
- [8] A. Salabat, F. Mirhoseini, F.H. Nouri, Microemulsion strategy for preparation of $\text{TiO}_2\text{-Ag}$ /poly(methyl methacrylate) nanocomposite and its photodegradation application. *J. Iranian Chem. Soc.* 20 (2022) 599–608. <https://doi.org/10.1007/s13738-022-02693-7>.
- [9] M. Hampel, J. Blasco, H. Segner, H. Molecular and cellular effects of contamination in aquatic ecosystems. *Environ. Sci. Pollut. Res.* 22 (2015) 17261–17266. <https://doi.org/10.1007/s11356-015-5565-5>
- [10] A. Salabat, F. Mirhoseini, M. Mahdieh, H. Saydi, A novel nanotube-shaped polypyrrole-Pd composite prepared using reverse microemulsion polymerization and its evaluation as an antibacterial agent, *New J. Chem.* 39 (5) (2015) 4109–4114. <https://doi.org/10.1039/c5nj00175g>
- [11] F. Mirhoseini, Alireza Salabat, Ionic liquid based microemulsion method for fabrication of poly(methyl methacrylate)- TiO_2 nanocomposite as highly efficient visible light photocatalyst, *RSC Adv.* 5 (2015) 12536–12545. <https://doi.org/10.1039/c4ra14612c>
- [12] A. Salabat, F. Mirhoseini, Applications of a new type of poly(methyl methacrylate)/ TiO_2 nanocomposite as an antibacterial agent and a reducing photocatalyst. *Photochem. Photobiol. Sci.*, 14(9) (2015) 1637–1643. <https://doi.org/10.1039/c5pp00065c>
- [13] A. Salabat, F. Mirhoseini, R. Valirasti, Engineering poly(methyl methacrylate)/ Fe_2O_3 hollow nanospheres composite prepared in microemulsion system as a recyclable adsorbent for removal of benzothiophene, *Ind. Eng. Chem. Research* 58 (2019) 17850-1785. <https://doi.org/10.1021/acs.iecr.9b04322>
- [14] S. Li, Zhang H, Cong B, He P, Liu W, Liu S. A Novel $\text{Ag}@\text{AgCl}$ Nanoparticle Synthesized by Arctic Marine Bacterium: Characterization, Activity and Mechanism. *Int. J.Mol. Sci.* 2022; 23(24):15558. <https://doi.org/10.3390/ijms232415558>
- [15] I.N. Savina, M. Zoughaib, A.A. Yergeshov, Design and assessment of biodegradable macroporous cryogels as advanced tissue engineering and drug carrying materials. *Gels.* 7(3) (2021) 79. <https://doi.org/10.3390/gels7030079>.
- [16] L.J. Eggermont, Z.J. Rogers, T. Colombani, A. Memic, S.A. Bencherif, injectable cryogels for biomedical applications. *Trends Biotechnol.* 38 (2020) 418–431. <https://doi.org/10.1016/j.tibtech.2019.09.008>.
- [17] S.-L. Loo, W.B. Krantz, T.-T. Lim, A.G. Fane, X. Hu, Design and synthesis of ice-templated PSA cryogels for water purification: Towards tailored morphology and properties. *Soft Matter* 9 (1) (2013) 224-234.
- [18] N.D. Trinh, T.T.B. Nguyen, T.H. Nguyen, Preparation and characterization of silver chloride nanoparticles as an antibacterial agent. *Adv. Nat. Sci. Nanosci. Nanotech.*, 6(4) (205) 045011. <https://doi.org/10.1088/2043-6262/6/4/045011>
- [19] X.N. Xiao, F. Wang, Y.T. Yuan, J. Liu, Y.Z. Liu, X. Yi, Antibacterial activity and mode of action of dihydromyricetin from *Ampelopsis grossedentata* leaves against food-borne bacteria. *Molecules* 24(15) (2019) 2831. <https://doi.org/10.3390/molecules24152831>
- [20] N. Tehri, A. Vashishth, A. Gahlaut, V. Hooda, Biosynthesis, antimicrobial spectra and applications of silver nanoparticles: current progress and future prospects. *Inorg. Nano-Metal Chem.* 52(1) (2020) 1–19. <https://doi.org/10.1080/24701556.2020.1862212>