Hybrid Nanocomposite Wound Dressings by a Novel Nanorod Vitamin-B3-Ag Metal-Organic Framework and Bacterial Cellulose Nanofibers

Mahdi Barjasteh  
Shahid Beheshti University

Seyed Mohsen Dehnavi (✉ mo.dehnavi@sbu.ac.ir)  
Shahid Beheshti University

Shahab Ahmadi Seyedkhani  
Sharif University of Technology

Mehrdad Akrami  
Shahid Beheshti University

Marzieh Rahimi  
Shahid Beheshti University

Article

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Abstract

This paper presents a novel nanorod metal-organic framework made of silver nods configured within nicotinic acid (vitamin-B3) linkers (AgNA) aimed at wound healing applications. X-ray diffraction analysis indicated that the synthesized AgNA MOFs comprised of zigzag chains of silver (I) nicotinate with orthorhombic crystal structure. Electron microscopy showed nanorod structures for the MOFs with uniform dimensions and element distribution. By seeding the AgNA MOFs into the bacterial cellulose (BC) nanofibers, an innovative BC-xAgNA nanocomposite was fabricated for wound dressing applications. MTT assay demonstrated improved biocompatibility for the BC-AgNA nanocomposites up to more than 116% cell viability. The Acridine Orange staining showed more than 87% of live/dead cells ratio for the prepared wound dressings. The fibroblast cells attached on the BC-AgNA nanocomposite exhibited expanded morphologies with long filopodia. The in vitro cellular scratch analysis demonstrated excellent wound healing by more than of 96% wound closure rate of the wound cured with the BC-AgNA nanocomposite. Evaluating the BC-xAgNA nanocomposites revealed their appropriate antibacterial activities against different bacterial strains. Synergistic wound healing effects corresponding to vitamin-B3, Ag, and BC nanofibers were observed. The results confirmed that the designed BC-AgNA nanocomposite can potentially be considered for wound healing and damaged tissue regeneration.

1. Introduction

Skin wounds are physical injuries that are sometimes accompanied by unpleasant side effects such as severe bleeding. However, in some cases, the wounds can be more catastrophic, which leads to microbial infections and ultimately death. Recent reports have revealed that bacterial infections origin ~ 13.6% (1 in 8) of all global deaths, which makes them the second-leading cause of death globally. The wounds provide possible and available pathways for infections to enter the body. Dressing the wound for healing and developing various processes that help accelerate wound healing are as old as human history. Today, wound dressings are advanced materials that are designed and manufactured with several purposes, including helping blood coagulation in the injured region \(^1\), preventing infections \(^2\), treating inflammation \(^3\), drug delivery \(^4,5\), accelerating the healing process \(^6,7\), and regeneration of the lost tissue \(^2,8\). Among the various properties of wound dressings, the ability to prevent bacterial infections is one of their most important characteristics. In this regard, various approaches, such as using antibacterial drugs, have been proposed \(^9,10\). However, the chemical drugs can result in bacterial drug resistance, and weakening of the patient's immune system \(^11\).

Recently, the nanocomposite wound dressing embedded with antibacterial materials, especially metallic nanoparticles, has become one of the interesting and developing approaches for treating wounds \(^12\). Many researchers have demonstrated more efficient healing process with nanocomposite dressings than traditional ones \(^13–15\). However, these nanoparticles can be surrounded by the proteins and other biological species present in the body, losing their effectiveness \(^16\). Agglomeration and subsequent
decentralized releasing of the nanoparticles can lead to unbalanced effects and could be other shortcomings of the wound dressings functionalized with the metallic nanoparticles.

Metal-organic framework (MOF) is known as a type of nanoporous materials consisting of metal ions or clusters that coordinate with organic ligands, which create nanoscale three-dimensional (3D) structures. The MOFs have various rising applications, including drug delivery systems, gas separation and storage, biomimetic mineralization, energy storage, and advanced tissue engineering. Moreover, having a fully nanoporous platform, the MOFs-functionalized wound dressings can mimic the biological nanoscale structures of the extracellular matrix (ECM). Silver-based MOFs (Ag-MOFs) are one of the new kinds of MOFs, which can be made using different organic ligands. Compared to the silver nanoparticles (AgNPs), the Ag-MOFs can act as a source for the gradual release of the Ag ions. Berchel and co-workers designed a three-dimensional (3D) Ag-MOF made of Ag$^{+}$ ions and 3-phosphonobenzoic acid, which effectively destroyed bacterial cells through the continuous release of the Ag$^{+}$ ions. While the toxicity of the Ag-MOFs to human red blood cells is negligible. It has been approved that the Ag-MOFs benefit from superior antibacterial effects on different bacterial strains, including E. coli strain MG1655, three S. aureus strains methicillin-resistance S. aureus (MRSA), RN4220 and Newman, and two Pseudomonas aeruginosa (PA) strains PA240709 and PA130709. Moreover, the Ag-containing materials can limit undesired biofilms formed by the Enterobacter cloacae, Streptococcus thermophiles, and Propionibacterium acnes, the bacteria that are generally existent within the wounds. Ning et al. synthesized various Ag-MOFs using different organic carboxylic acid ligands. They demonstrated that the production of antibacterial properties was mainly related to the slow Ag$^{+}$ releasing, and synergistic effect associated with the reactive oxygen species (ROS). Therefore, it would be appropriate to mix various tissue repair approaches with the MOFs to develop a multi-functional wound dressing equipped with histocompatibility for treatment of the wound infections.

In order to design and produce efficient wound dressings, recognizing the biological and structural characteristics of the target living tissue is critical. The ECM is a complex network made of interwoven nanoscale fibrous proteins that in addition to supporting the cells, can direct the cell activities through creating the guiding platforms. Therefore, it is difficult to design and build scaffolds that can satisfactorily mimic the ECM's behaviors. Using the synthetic nanofibers to biomimetic the ECM's polymeric network along with the addition of favorable components such as the MOFs for inducing desired properties, has led to development of the novel wound dressings. Accordingly, in this research, nanofibrous bacterial cellulose (BC) was used as the matrix of the wound dressing to create a porous structure with a high specific surface area, creating pathways for oxygen and body fluids, along with mimics the nanoscale ECM tissues. An innovative nanorod silver-based MOF (AgNA) was fabricated using biocompatible niacin (the active form of vitamin-B3) ligands. The niacin benefits from optimistic properties, including antioxidant, anti-inflammatory, and immunomodulatory activities, as well as epithelization-inducing action, which can offer positive effects on the wound healing process. The AgNA MOFs were synthesized through an environmentally-friendly, simple, and cost-effective process. The effects of adding prepared AgNA MOFs on the physico-chemical, structural, and biological performances
of the BC wound dressings were investigated. According to our best knowledge, this kind of multicomponent biological nanocomposites, i.e. the bacterial cellulose nanofibers functionalized with the AgNA MOFs, has not been created for wound dressings. Therefore, it is expected that the present study will create new perspectives for the development of the porous, biomimetic, and multifunctional platforms so that they can make constructive interactions with the living tissue, and heal the wounds in shorter periods of time.

2. Experimental procedures

2.1. Materials

Nicotinic acid \( (C_6H_5NO_2) \) that is also known by the names of niacin or vitamin-B3), silver acetate \( (AgC_2H_3O_2) \), and ethylene glycol were purchased from the Merck (Germany). Bacterial cellulose nanofibers gel was supplied from Nano Novin Polymer (I.R.Iran). Dulbecco’s Modified Eagles Medium (DMEM) was purchased from Gibco (USA). Trypsin and Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (Germany). Fetal bovine serum (FBS) was provided from Gibco (USA). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was supplied from Cal Roth (Germany). Fibroblast cells (L929, rat strain) were supplied from the Iranian Biological Resource Center (IBRC). The Staphylococcus aureus \( (S. aureus, \text{ATCC } 25923) \) and Escherichia coli \( (E. coli, \text{ATCC } 25922) \) bacterial strains were used as Gram-negative and Gram-positive model systems, respectively. Highly pure deionized water (DIW) was used for creating all aqueous solutions. All compounds used in this study were analytical grade and were used with no additional purification.

2.2. Synthesis of Nanorod AgNA MOFs

0.123 mg of nicotinic acid was dissolved in a solution comprising 15 ml of DIW and 5 ml of ethylene glycol (solution A). Separately, 1 mmol of silver acetate \( (AgC_2H_3O_2) \) was dissolved in a solution comprising 15 ml of DIW and 5 ml of ethylene glycol (solution B). Solution A was stirred at 80°C for 15 min. Then, solution B was rapidly poured into solution A, which was stirred at high speed. The mixture of solutions was stirred for an additional 15 min to make a homogenous solution and complete the desired reactions. The obtained solution was centrifuged at 7000 rpm. The precipitates were washed twice by 60°C DIW. After that, the obtained products were washed with ethanol and finally dried at ambient temperature.

2.3. Fabrication process of BC-AgNA nanocomposite

For fabricating the BC-AgNA nanocomposites, an aqueous solution containing 0.5 wt.% BC fibers were prepared. The solution was stirred for 10 min to make a homogenous suspension. After that, different AgNA MOF amounts were added into the separate suspensions that were being stirred, to make BC-xAgNA nanocomposite suspensions \( (x: \text{AgNA to BC weight ratio} = 0.1, 0.25, 0.5, \text{and } 1) \). The BC-xAgNA suspensions were mixed with 30 min additional stirring to make monodisperse blends. The prepared blends were separately poured into 24-well flat microplates, and freeze-dried.
2.4. Analysis & Characterization

The samples' functional groups were characterized by the Fourier transform infrared (FTIR) spectroscopy with a resolution of 6 cm$^{-1}$. The crystal phases of the synthesized samples were examined by x-ray diffraction (XRD) using Cu K$_{\alpha}$ radiation. Field-emission scanning electron microscopy (FE-SEM) equipped with energy-dispersive x-ray spectroscopy (EDX) was used to study the morphology and element mapping analysis of the samples.

2.5. Biological tests

Cell culture procedure, biocompatibility measurements by the MTT assay, and cell attachment studies were described in detail in our previous works$^{2,3}$. Briefly, the fibroblast cells (L929) were supplied from the Iranian Biological Resource Center (IBRC). After the cell culture process, the MTT assay was conducted by seeding ~ 5000 cells into each sample. Then, the samples were incubated at 37°C for 72 h. After that, 1 ml of MTT solution was added into each sample, followed by incubation of the samples for 4 h. In the next step, the formazan crystals dissolved in the DMSO, and finally the cellular optical densities were calculated at ~ 570 nm using the Elisa reader spectroscopy. For cell attachment, ~ 5000 fibroblast cells were added to each sample. The samples were incubated for 24 h. Then, the cells were washed by 4% paraformaldehyde and PBS, respectively. Next, the samples were incubated with glutaraldehyde (C$_5$H$_8$O$_2$) at ambient temperature, washed with PBS and dehydrated using alcohol. Finally, a thin layer of gold (Au) was applied on the samples’ surfaces by sputtering for FE-SEM studies.

2.5.1. Acridine Orange (AO) staining

The AO staining was performed by seeding the cultured fibroblast cells to the prepared wound dressing. Next, the AO solution (Sigma-A9231) was poured onto the cells. The samples were kept at ambient temperature for 15 min. After that, the samples were washed three times with PBS. After that, the propidium iodide (PI, Sigma-P4864) was added to the cells, followed by several washing processes with PBS. Finally, the samples were studied using a fluorescent microscope.

2.5.2. Scratch test

Scratch analysis was performed by adding ~ 50000 of the fibroblast cells into each well of a 24-wells plate. The cells were allowed to adhere to the bottom of the plate and proliferate. After the cell density reached about 90%, a scratch was made in the center of each well. Subsequently, the scratch regions treated with the fabricated wound dressings were studied after 0, 24, 48, and 72 h of the scratching. An optical microscope (with 40 magnification) was used for evaluating the scratches. Finally, the results were analyzed using the ImageJ software.

2.6. Antibacterial activities

The samples' antibacterial activity was examined via the agar diffusion technique using different bacterial strains of *E. coli* and *S. aureus* cells. For the first step, ~ 100 µl of each bacteria medium was
poured into each plate, and subsequently, the plates were incubated. The agar was contacted with the same surface of each sample. Next, the plates containing the agar and samples were incubated at 37°C for 24 h. Finally, the antibacterial performance of each sample was evaluated based on the formed inhibition zone around it.

3. Results and discussion

3.1. Physico-chemical analyses of the AgNA Nanorods

Figure 1a shows the XRD patterns of the experimental synthesized and simulated AgNA MOFs. As is seen, the XRD analysis for the synthesized AgNA MOF indicated a high agreement with the simulated results and data reported in the previous works. Accordingly, the fabricated AgNA MOF was the silver (I) nicotinate \((\text{C}_5\text{H}_4\text{NCO}_2\text{Ag})\) with an orthorhombic crystal structure. Figure 1b shows the crystal structure proposed for the fabricated AgNA MOF, showing zigzag chains comprising of the Ag atoms (yellow) with \(d_{\text{Ag}-\text{Ag}} \approx 3.034 \, \text{Å}\). The crystal is a 3D network in that each Ag node was fivefold linked, including two oxygen (red) related to distinct nicotinate ligands \((\text{Ag-O} \approx 2.258 \text{ and } 2.272 \, \text{Å})\), one nitrogen of a third ligand \((\text{Ag-N} \approx 2.359 \, \text{Å})\), and two other Ag atoms, showing a dissymmetric trigonal bipyramid.

Chemical bonds and functional groups of the synthesized AgNA MOFs were evaluated by FTIR analysis, as indicated in Fig. 1c. The vibrational peaks within 3070 – 2805 cm\(^{-1}\) were related to C-H stretching. The peaks that appeared near 1595 cm\(^{-1}\) and at 1695–1709 cm\(^{-1}\) were assigned to C = C stretching and C = O (COO\(^{-}\)) asymmetrical stretching, respectively. Absorption peaks near 1415, 1322, and 1300 cm\(^{-1}\) were assigned to C = N symmetric stretching, C = O symmetrical stretching, and C-N stretching, respectively. The C-O stretching was revealed by the peak appeared at 1185 cm\(^{-1}\). Moreover, the peaks that appeared within 1032–1115 cm\(^{-1}\) and 643–811 cm\(^{-1}\) could be assigned to C-H in plane and out plane bending vibrations, respectively. It is worth noting that the FTIR result for the synthesized AgNA was well supported by the previous works.

Figure 1d and e illustrate the FE-SEM image and EDS mapping analysis of the AgNA MOFs. As is observed, the prepared AgNA MOFs have highly uniform nanorod morphologies with narrow dimension distribution. The kind of distribution and uniform dimensions can be appropriate properties during the AgNA MOFs are applied as reinforcement to fabricate monodisperse composites. Furthermore, the uniform characteristics could help the platforms for controlled releasing processes when the AgNA MOFs were used as drug/molecule/gene carriers. On the other side, the AgNA MOFs showed nanorod morphologies having a proper aspect ratio for excellent interlocking into the nanofibrous BC matrix, resulting in mechanical stability. Moreover, the one-dimensional (1D) nanorod morphology with sharp edges could be preferred places for the accumulation of electric charges that affect the mechanisms of protein adsorption at tissue-biomaterial interfaces. The EDS mapping analysis revealed that the synthesized MOFs were mainly comprised of silver (Ag), carbon (C), nitrogen (N), and oxygen (O) atoms.
As is seen, the AgNA MOFs benefited from a uniform distribution of Ag, C, and N atoms across entire of the nanorods.

### 3.2. BC-AgNA Nanocomposites

The XRD patterns and FTIR analysis of the fabricated pure BC and BC-AgNA nanocomposite have been indicated in Figs. 2a and b, respectively. The XRD demonstrated the main characteristic peaks of the bacterial cellulose at ~ 15, 17, and 22.5° that were assigned to (100), (010), and (110) crystal planes, respectively. The obtained phases were in accordance with the phases of the bacterial cellulose produced by *Gluconacetobacter xylinus* strains ATCC 53524 and ATCC 23768, which were reported in previous studies. While, the main peaks obtained for the AgNA MOFs, as shown in Fig. 1a, remained in the BC-AgNA nanocomposite sample. The FTIR study of the BC-AgNA showed absorption peaks corresponding to the bacterial cellulose and AgNA MOFs simultaneously. In addition to the peaks assigned to the AgNA MOFs, which were discussed in Fig. 1b, the nanocomposite showed the BC's characteristic peaks near 2800, 1672, 1488, and 1225 cm\(^{-1}\) that attributed to the –OH, –CH\(_2\), –C=O, and –C–O–C bonds, respectively. Accordingly, it could be found that the chemical and structural properties of the synthesized AgNA MOFs have been preserved during the fabrication process of the nanocomposites, and the prepared wound dressings contain each of the desired components.

The FE-SEM micrographs of the nanofibrous pure BC matrix and BC-AgNA nanocomposite have been shown in Fig. 2c-e. As is observed, the BC matrix was made of interwoven nanoscale fibers, showing monotonous morphology with high coherency and uniform dimensions. Moreover, the nanofibrous BC platform supplies extensive porosities that serve as appropriate pathways for biological nutrients, fluids, and oxygen transferring accompanied by great water storage capabilities. On the other side, the FE-SEM images of the BC-AgNA nanocomposite exhibited uniform AgNA MOFs distribution within the polymeric nanofibers configuration. As is observed, the AgNA MOFs were mechanically interlocked by the BC nanofibers, preventing the undesired release of the MOFs. This interlocking can lead to maintaining the nanocomposite's structural coherency and mechanical stability. Hence, in addition, to slow Ag\(^+\) releasing during degradation of the AgNA MOFs, an additional hierarchical releasing mechanism of the BC-covered AgNA MOFs can be occurred through the BC fibers decomposition (see Fig. 2e). In other words, by the BC fibers decomposition, the AgNA MOFs attached on the fibers, released and diffused into the biological fluid. Where the released AgNA MOFs can be degraded far from the wound dressing's surface. Therefore, antimicrobial agents such as Ag\(^+\) species, which might be coagulated by the bacterial cells and lose their effectiveness, travel longer distances to inhibit bacterial activities and infections. The EDS analysis approved the existence and uniform distribution of the AgNA MOFs within the BC matrix, as shown in Fig. 2f. This kind of monotonous blending allows the AgNA MOFs to be in contact with the living tissue. Compared to the silver nanoparticles (AgNPs), the AgNA MOFs can serve as the reservoirs for the gradually release of the Ag species for long-term antimicrobial purposes, and the vitamin-B3 as the bioactive agents. Therefore, the BC-AgNA nanocomposites could be appropriate biomaterials to control the wound healing processes.
3.3. Biological assays

3.3.1. Cell viability and biocompatibility

The biocompatibility of the samples was assessed via the MTT test. The results were described as the cell viability values, as indicated in Fig. 3. A control sample with 100% of the cell viability was considered and other measurements were normalized to it. As is seen, all prepared samples demonstrated excellent biocompatibility with high cell viability indexes. As expected, the pure BC indicated proper cell viability of 98% due to its intrinsic biocompatibility. While it was interesting that the produced BC-AgNA nanocomposites showed extraordinary improvements in the cell viabilities by adding the AgNA MOFs. The nanocomposites containing 10 and 25 wt.% of AgNA MOFs indicated more than 100 and 107% of cell viability values, respectively. Moreover, the BC-50AgNA nanocomposite showed the best biocompatibility with approximately 116% of cell viability, demonstrating more than 18% improved cell viability in comparison with the pure BC. The cell viability of the BC-100AgNA decreased a little compared to the other nanocomposites, although its biocompatibility remained within an appropriate range for biomedical application. It should be noted that the obtained decrease in biocompatibility of the BC-100AgNA could be related to the intemperate AgNA concentration. Previous works have indicated that Ag can introduce cell toxicity and DNA destruction. Hence, excessive Ag concentrations may lead to cell damage. Furthermore, the niacin base 3D structure of the AgNA MOFs is talented to be degraded in aqueous environments, resulting in extra releasing of the Ag that attacks the cells. On the other side, the vitamin-B3 has positive biomedical effects, including anti-apoptotic, antioxidant, and anti-inflammatory properties that can encourage cell viability. Hence, it can be found that there is a competition between the positive effects corresponding to release of the vitamin-B3, and the adverse effects due to high Ag concentrations, which determine the ideal characteristics for the nanocomposites. Accordingly, an optimum AgNA MOFs amount should be considered. In this regard, the BC-50AgNA could be a promising candidate; however, complementary investigations need to be considered.

3.3.2. Acridine Orange staining

For more detailed investigations of the cellular responses at the tissue-biomaterials interface, the Acridine Orange (AO) staining was used. The AO is a metachromatic staining that determines the cell cycle by selecting the nucleic acids. The AO can interact with deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) through the intercalation process and electrostatic attraction, respectively. During the staining, the DNA intercalated the AO fluoresces green (~ 525 nm), and the RNA electrostatically bound the AO fluoresces red (> 630 nm). Hence, it can cause differences between activated, quiescent, and proliferating cells. Figure 4 illustrates fluorescent microscopy images of the live and dead cells for the control, pure BC, and BC-50AgNA nanocomposite samples. The pure BC and the BC-50AgNA demonstrated significant live cells to dead cell ratio (LDR) of 90 and 87.3%, respectively. While the control sample showed an approximately ~ 94% LDR. As is seen, the fabricated wound dressings benefited from high LDRs, which approved the proper biocompatibility measurements.
3.3.3. Cell attachment and morphologies

Figure 5 shows FE-SEM images of the cells’ morphologies spread onto the pure BC nanofibers and the BC-AgNA wound dressings. The images indicated proper cell attachment on the fabricated surfaces, demonstrating eligible interactions between the cells and produced platforms. Moreover, the cells attached to the BC-AgNA nanocomposite indicated expanded morphologies with long filopodia, as shown in Figs. 5e and f.

These prominent performances for the BC-AgNA nanocomposite could be attributed to (i) nanoscale roughness by the BC nanofibers and the nanorod AgNA MOFs that mimic the ECM tissue\(^\text{43–45}\), (ii) synergistic effects of the Ag and vitamin-B3 that can modulate cell energy\(^\text{46}\), (iii) bridging force mechanisms between biological species and the biomaterial's surface\(^\text{47}\), accompanying with (iii) highly porous platform of the nanofibrous matrix that provides proper pathways for biological fluids, nutrient storage, and oxygen penetration\(^\text{48}\).

3.3.4. Cell migration and wound healing

The scratch test is an \textit{in vitro} method that evaluated the cellular and molecular processes to determine the cell migration mechanisms. This technique can also be applied to study therapeutic agents prior to clinical applications. Quantitative results obtained from the scratch assay, which represent the scratch closure rates by the fabricated wound dressings at different post-treatment times, have been indicated in Fig. 6. The results measured for the control samples after 24, 48, and 72 h of treatment revealed approximately 29.74, 43.65, and 56.8\% of the scratch closure rates, respectively. The closure rates of the scratches treated using the pure BC were calculated 39.46, 49.37, and 65.59\% from 24 to 72 h of the treatment. However, the highest scratch closure rates were obtained for the wounds treated by the BC-50AgNA nanocomposite. The fabricated nanocomposite led to excellent scratch closure rates of 96.61\% at 72 h of post-treatment. Moreover, the initial analysis revealed appropriate values of 62.08 and 80.58\% of scratch closure rates after 24 and 48 h, respectively, which were two times greater than the rates measured by the other samples. The measurements demonstrated 47–63\% improved scratch closure rates by adding the synthesized AgNA MOFs to the BC nanofibers. The obtained results were in agreement with the previous reports on the improvement of the wound healing process by adding Ag and Vitamin-B3 containing compounds\(^\text{3,40,49}\).

It has been demonstrated that vitamin-B3 offers immunomodulatory effects along with the ability to modulate cell energy\(^\text{46}\). In addition, this vitamin can inhibit endothelial mesenchymal transition and accelerates wound healing\(^\text{50}\). On the other side, by enhancing the proliferation and migration of keratinocytes, the Ag-containing compounds can increase the wound closure rates\(^\text{51}\). Many investigations have demonstrated that the platforms functionalized with Ag species can induce improved cell attachment and spreading at the initial stages of the cell culture, compared to those Ag-free platforms\(^\text{52}\). Moreover, it is worth noting that the Ag nanoparticles can differentiate the fibroblast cells
into myofibroblasts, which provide tractive forces to pull the wound's edges together. The wound closure mechanisms by the myofibroblasts could be described based on two hypotheses: (i) the tensile forces are carried out during migration of the fibroblast cells to the ECM tissue, resulting in compression of the ECM and consequent wound closure. The next hypothesis states that (ii) the myofibroblasts can attach to ECM and act as the same smooth muscle cells. Although, recently it is widely agreed that both mentioned mechanisms can operate simultaneously. The combined theory assumes that the wound closure process includes four steps: (1) the fibroblast cells attached to collagen fibers exert tensile forces on the ECM. (2) These forces lead to initial compression that activates healing processes. (3) The applied compaction along with microenvironmental changes cause differentiation of the fibroblast cells to myofibroblasts. Finally, (4) the myofibroblasts cells proliferate, making significant tractive forces at the wound region and repairing the damaged tissue. Figure 7 illustrates optical microscopy images of the scratches treated using the prepared wound dressings.

### 3.3.5. Antimicrobial tests

Figure 8 shows the antibacterial activities of the fabricated wound dressings measured via the Agar analysis by using two bacterial strains of *E. coli* and *S. aureus*. As expected, the BC sample indicated no effective antibacterial performance against the bacteria, which could be attributed to its intrinsic bio-inert characteristic. While, the inhibition zones were visible for the samples containing AgNA MOFs so that they increased with the increase in the MOF concentration. This appropriate activity was observed against both used Gram-positive and Gram-negative bacteria, demonstrating efficient antibacterial properties of the produced BC-AgNA nanocomposites. However, it should be noted that the different antibacterial performances against the studied bacteria could be originated from different causes, such as less soft membrane of the *E. coli*, which were in accordance to the previous works. Hence, it could be found that the antibacterial activity of the nanocomposites is related to the AgNA MOFs. Many studies have discussed the antimicrobial activity mechanisms of Ag-containing materials. By a general agreement, the Ag ions can attack to the bacterial cell by attaching to its membrane's proteins. This attachment is performed because of the high affinity of the Ag species towards the sulfur-containing proteins of the membrane, which changes and destroys the bacterial cell's structure. This phenomenon proceeds via condensing and banning the DNA replication by the Ag, resulting in the formation of chemical complexes. The produced complexes allow the Ag ions to bind to the protein's thiol moieties and disrupt the respiratory enzyme's activities. Accordingly, the surface free radicals formed on the Ag can make antibacterial properties.

### 4. Conclusions

New nanocomposites comprising of a novel Ag-vitamin-B3 MOF and bacterial cellulose nanofibers, were produced for wound healing applications. The MOF was synthesized through a green method using environmentally friendly inexpensive materials. The results indicated appropriate biocompatibility of the nanocomposite and improved cell viability by adding the produced MOFs, which indicated a competitive
performance between positive effects of vitamin-B3 and cytotoxicity related to the excessive concentration of Ag. Expanded morphologies with long filopodia of the cells spread on the BC-AgNA samples demonstrated the potential of the nanocomposite dressings to support cellular activities. The cellular scratch analysis revealed outstanding healing for the wounds treated with the BC-AgNA nanocomposites. Moreover, the produced nanocomposites showed antibacterial properties against both Gram-negative and Gram-positive bacterial strains. This study can facilitate the development of the future fully porous platforms consisting of bioactive agents capable to encourage wound healing within a short time period.

Declarations

Data Availability

The data that support the findings in this study are available from the corresponding author upon reasonable

References


24. Rahnamaee, S. Y. *et al.* Boosting bone cell growth using nanofibrous carboxymethylated cellulose and chitosan on titanium dioxide nanotube array with dual surface charges as a novel


**Figures**

![Figure 1](image)

**Figure 1**

(a) Experimental and simulated XRD patterns and (b) crystal structure of the AgNA MOFs [silver (yellow), carbon (gray), oxygen (red), and nitrogen (blue)]. (c) FTIR spectrum of the synthesized AgNA Nanorod MOFs. (d) FE-SEM image of the synthesized AgNA nanorods (inset: scale bar is 5 µm) and (e) corresponding EDX mapping analysis.
Figure 2

(a) XRD and (b) FTIR analyses of the fabricated bacterial cellulose (BC) and BC-AgNA nanocomposite. FE-SEM micrographs of (c) pure BC (inset: higher magnification image of the nanofibers, scale bar is 500 nm), (d) BC-AgNA nanocomposite (inset: higher magnification image of the nanocomposite, scale bar is 1 µm. (e) High magnification FE-SEM image of the AgNA MOFs covered by the BC nanofibers. (f) Electron microscopy and related EDX mapping analysis of the BC-AgNA nanocomposite, showing silver (yellow), carbon (dark pink), and oxygen (light blue) elements (scale bars are 5 µm).
Figure 3

Cell viability measurements obtained from the MTT assay for the control, bacterial cellulose (BC), and the BC-xAgNA nanocomposites containing different AgNA concentrations (x: 10-100 wt.%).
Figure 4

Acridine Orange (AO) staining showing the live and dead cells on the control, pure BC, and BC-50AgNA nanocomposite. Green and orange regions represent the live and dead cells, respectively (To see the colors and precise details of this figure, the reader is referred to the web version of the article).
Figure 5

FE-SEM micrographs of the fibroblast cells attached and growing with different morphologies on (a-d) pure nanofibrous BC and (e-h) BC-AgNA nanocomposite platforms. (e, f) The fibroblast cells show expanded morphologies with long filopodia on the fabricated BC-AgNA nanocomposites. (g, h) The cells attached properly on the BC nanofibers and AgNA MOFs simultaneously.

![Bar chart showing scratch closure rates](chart.png)

Figure 6

Scratch closure rates after 24, 48, and 72 h of treatment by the control, pure BC nanofibers, and the BC-50AgNA nanocomposite.
Figure 7

Optical microscopy images of the cellular scratches after 0, 24, 48, and 72 h of treatment using the control, BC, and BC-50AgNA wound dressings.
Figure 8

Antibacterial performances evaluated by the Agar diffusion test. Inhibition zones formed by the fabricated specimens against *E. coli* and *S. aureus* bacteria as the Gram-negative and Gram-positive strains, respectively.