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Manuscript Number: MSEC_2020_1651R1

Fabrication of cellulose carbamate hydrogel-dressing with rarasaponin surfactant for enhanching adsorption of silver nanoparticles and antibacterial activity

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Author's Response To Reviewer Comments

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Editor and Reviewer comments:
Reviewer #1:
In the present work, the authors reported an evolutionary progress in terms of strategies to increase the loading of AgNP into cellulose carbamate hydrogels by utilizing surfactants. Overall, the article is well organized and its presentation is good. However, some minor issues still need to be improved:

(1) Although, according to the data on Ag loading capacity (LC), CCH-R has the highest LC values compared to CCH-T and CCH-C, it is found that CCH-C has the highest antibacterial activity, followed by CCH-R > CCH-T > CCH, from the results of ZOI measurements. How to explain the relationship between the Ag loading capacity and the antibacterial activity?
Response: We have added the explanation regarding the relationship between the Ag loading capacity and the antibacterial activity as suggested by the reviewer. Please refer to P26-27 L433-442 in the revised manuscript.
P26-27 L433-442: This released AgNP then damages bacterial cells by inducing the formation of hydroxyl radicals and penetrate into/interferes with DNA replication through the damaged cells, and thereby causing cell death. The more AgNPs come into contact, the damaging effect to the bacterial cells will be exaggerated. From the results of ZOI measurements, it was found that CCH-C had the highest antibacterial activity, followed by CCH-R > CCH-T > CCH. This is because the presence of surfactants helps increase the loading of AgNPs so that antimicrobial activity also increases. Distinct to other systems, in CCH-C—Since CTAB itself possess an antibacterial activity (as observed in negative control); thus the antibacterial activity does not solely depend on the amount of AgNPs in its matrix but also due to the presence of CTAB.

(2) The antibacterial activity of AgNPs@CCH is significant. From in vitro cell cultivation tests, AgNPs have been reported to be toxic potential to several human cell lines. Should biocompatibility and cytotoxicity be evaluated in this work?
Response: We have evaluated the cytotoxicity of the prepared hydrogels with AgNPs loading as suggested by the reviewer. Please refer to subsection 3.5 in P25 L414-419 in the revised manuscript.
P25 L414-419: The cytotoxicity evaluation is an extensive way of demonstrating the biocompatibility of the prepared hydrogel as a wound dressing material. Fig. 9 shows that none of the remaining cell viability was lower than 80%. According to ISO 10993-5: 1999, no cytotoxic potential in samples with cell viability higher than 70%. Therefore, it can be concluded that the AgNPs loaded hydrogels can maintain the normal function of skin fibroblasts. It can also be noted that the use of surfactant higher than CMC did not exhibit toxicity even in the high concentration of 10 mM.

(3) Elemental maps of the distribution of AgNPs is clear and important. The authors should provide elemental maps of Ag on CCH-T and CCH-C in Fig 6.
Response: We have provide the elemental maps of Ag on CCH-T and CCH-C as suggested by the reviewer. Please refer to Figure 6c in the revised manuscript.

(4) To clarify the antibacterial mechanism, the authors need to clarify how AgNPs disperse from hydrogels and come into contact with bacteria.
Response: We have added the explanation regarding the relationship between the Ag loading capacity and the antibacterial activity as suggested by the reviewer. Please refer to P26 L429-435 in the revised manuscript.
P26 L429-435: The antibacterial mechanism of AgNPs has been reported in many studies [69, 70]. Briefly, Ag, which is a soft acid, tends to interact with soft bases, here, sulfur contained in protein membranes and phosphorus in bacterial DNA. Since soft acid-soft base interaction is preferable than the interaction of AgNPs with the hydroxyl groups of cellulose hydrogel, thus AgNPs will be released from the hydrogel. This released AgNP then damages bacterial cells by inducing the formation of hydroxyl radicals and penetrate into/interferes with DNA replication through the damaged cells, and thereby causing cell death.

(5) Page 18, line 304: "Fig. 6a" rather than "Figs. 6a". Page 20, line 347: "Fig. 8" rather than "Figs. 8".
Please carefully check the manuscript before submission.
Response: We have changed the words and corrected many typos of the revised manuscript.

Reviewer #2:
The manuscript reported the synthesis of cellulose carbamate (CC) loaded with silver nanoparticles (AgNPs) using sodium borohydride as a reducing agent. The authors further studied the effect of adding surfactants in promoting the loading of AgNPs. Overall, the authors adequately incorporated in this manuscript with all results in terms of preparation and characterization using various characterization techniques with sufficient explanations. Finally, the Antibacterial test results also confirmed the AgNPs@CCH (modified with surfactants) have better antibacterial activity towards the tested bacterial strain. Although many reports published on hydrogels loaded with AgNPs using various reducing agents such as sodium borohydrides, citric acid, hydrazine, natural extracts, and glycols, the paper is interesting to readers of MSEC.

Before acceptance of publication, the authors need to be addressed the following comments.

(1) The authors have explained the effect of the surfactant can increase the loading of AgNPs in the CCH using NaBH4 reducing agent. Some papers have reported the effect of functional groups on the hydrogel network could enhance the AgNPs loading. Thus the authors should explain the importance of present work in the introduction part with the other published papers.
Response: We have added importance of present work in the introduction part as suggested by the reviewer. Please refer to P5 L87-94 in the revised manuscript.
P5 L87-94: A new-facile approach in the incorporation of AgNPs to hydrogel was introduced. That is, by employing adsorption techniques of Ag+ ions onto the surface active sites of the hydrogel, followed by in situ reductions of the attached Ag+ ions. This approach allows the binding of large numbers of AgNP to the surface of the hydrogel dressing, thereby enhancing the antibacterial activity against the target bacteria. Furthermore, this approach could be an improvement over the conventional AgNPs hydrogel-dressing—wherein, in the preparation, AgNPs are integrated into the hydrogel matrix. So, the effectiveness of AgNPs in the center of the hydrogel matrix is reduced due to the difficulty of reaching the target bacteria.

(2) In general, tween80, it can act as reducing and stabilizing agents for the production of metal salts to metal nanoparticles. Thus, the silver nanoparticles can form without using NaBH4, please justify. (doi: 10.1186/1556-276X-7-612)
Response: Although Tween80 is reported to be oxidized in the presence of silver ions and act as reducing agent to synthesize AgNP, based on
the reaction happens at 90°C or higher temperature.
In this study, all reactions were done at room temperature thus Ag+ ions loaded with CCH-T were not reduced as indicated by the absence of hydrogel color changes, because the temperature has been known to play a role in the formation of AgNPs.

(3) In conclusion, the authors mentioned the developed AgNPs@CCH could be applied in wound dressing. However, toxicity is a major concern for particular biological applications. For this, the authors should incorporate cytotoxicity test results for all hydrogels, so that the conclusion well support with results.
Response: We have evaluated the cytotoxicity of the prepared hydrogels with AgNPs loading as suggested by the reviewer. Please refer to subsection 3.5 in P25 L414-419 in the revised manuscript.
P25 L414-419: The cytotoxicity evaluation is an extensive way of demonstrating the biocompatibility of the prepared hydrogel as a wound dressing material. Fig. 9 shows that none of the remaining cell viability was lower than 80%. According to ISO 10993-5: 1999, no cytotoxic potential in samples with cell viability higher than 70%. Therefore, it can be concluded that the AgNPs loaded hydrogels can maintain the normal function of skin fibroblasts. It can also be noted that the use of surfactant higher than CMC did not exhibit toxicity even in the high concentration of 10 mM.

(4) The citations were a little bit inadequate; I suggest some of the articles.
https://doi.org/10.1016/j.matlet.2016.08.043 https://doi.org/10.1002/pi.4789 https://doi.org/10.1002/jbm.a.34991
Response: We have added some references as suggested by the reviewer.

Reviewer #3:
This work (MSEC_2020_1651) demonstrated the antibacterial activities of Ag/carbamate cellulose nanocomposite hydrogels prepared by using three different surfactants. CCH-R sample showed the highest Ag loading and best antibacterial property. How about improve the Ag loading by using higher concentration of AgNO3 solution?
Response: We thank the reviewer for the comments. In this work, the amount of AgNO3 added during the loading is already excessive which is 106.28 fold higher than the highest LC that can be achieved by CCH-T10. Therefore, the use of higher concentration would not contribute to the increase of LC.

What is the effect of surfactants for the distribution and size of Ag nanoparticles?
Response: In order to know the effect of surfactant on the distribution we provide the elemental maps of Ag on hydrogels in Figure 6 in the revised manuscript. The size of AgNPs were determined by using TEM analysis as shown in Figure 7 in the revised manuscript. The related discussion has been added in P22 L377-385.
P22 L377-385: The effect of surfactant on the morphology of AgNPs has been characterized. As seen in the TEM image in Fig. 7, the resultant AgNPs exhibit spherical nano-size. The histogram depicts the particle size distribution of the synthesized AgNPs@CCH with and without surfactant modification with a mean particle diameter of 11.07±1.39, 9.52±0.28, 8.87±0.14, and 8.38±0.11 for CCH, CCH-T, CCH-C, and CCH-R, respectively. The AgNPs synthesized in surfactant modified CCH shows the smaller size and narrower particle size distribution than in the unmodified CCH. This may be due to aggregates remains in existence in the AgNPs prepared in the CCH without surfactant inside. A noticeable result obtained in the CCH-R that shows a significantly high relative frequency in the 5 to 10 nm range indicates a more uniform particle size.

What is the potential application of this material? wound dressing? Therefore, some issues should be responded before publication.
Response: The studied materials are potential as wound dressing, it has good antibacterial activities towards E. coli and S. aureus. Also, based on cytotoxic study, we have demonstrated the non-toxic behavior of the materials to the normal

cells.

Specific comments
1. Some related references about cellulose-based hydrogels with antibacterial activity should be included in the introduction of the manuscript, such Carbohydrate Polymers 2016, 137, 59
Response: We have added some related references regarding the cellulose-based hydrogels with antibacterial activity in the introduction as suggested by the reviewer. Please refer to P4 L67-68 in the revised manuscript.

2. Author are suggested to show the advantage of carbamate cellulose used here in comparison with other cellulose derivatives for the fabrication of cellulose-based hydrogels.
Response: We have added the superiority of cellulose carbamate as suggested by the reviewer. Please refer to P4-5 L77-86 in the revised manuscript.
P4-5 L77-86: Various dissolution methods have been established to resolve this limitation, such as the viscose process, carbamation, lyocell process, and the use of the ionic-liquid system [37]. Carbamation of cellulose to produce cellulose derivatives (i.e., cellulose carbamate) is known as an environmental dissolution method of cellulose compared to another soluble cellulose derivative (i.e., cellulose xanthogenate) which the synthesis accompanied by the generation of hazardous byproducts. Cellulose carbamate (CC) has better solubility in alkaline and organic solution compared to cellulose [38]. Furthermore, CC reported to possess bacteriostatic properties, which make it more resistant against microbial contamination and enzymatic-cleavage compare to cellulose. Gan et al. also reported that CC-based hydrogel has an enhanced porosity compare to cellulose hydrogels [39], and therefore, in this work, the carbamation process was chosen to dissolve the cellulose.

3. What is the degree of substitution of carbamate cellulose?
Response: We have added the degree of substitution (i.e., 0.34±0.03) of the synthesized cellulose carbamate as inquired by the reviewer. Please refer to P12 L257-259 in the revised manuscript.
P12 L257-259: From the results, it was found that CC5, with the ratio of cellulose to urea 1: 5, has the best transparency with a degree of substitution of 0.34±0.03 as calculated using the published equation [48] so that this ratio was chosen for further studies.

4. For the formation of hydrogels and the crosslinking of cellulose derivatives, some classic references should be cited instead of [48], such Macromolecular Bioscience 2007,7, 804; European Polymer Journal 2010, 46, 92; Macromolecules 2011, 44, 1642.
Response: We have added some references regarding the formation of hydrogels and the crosslinking of cellulose derivatives as suggested by the reviewer.

5. TEM is a useful method to investigate the size and distribution of silver nanoparticles in the hydrogel matrix, which will be benefit to understand the role of surfactants.
Response: We have added the TEM results to better understand the role of surfactant to the size of AgNPs. Please refer to Figure 7 and discussion P22 L377-385 in the revised manuscript.
P22 L377-385: The effect of surfactant on the morphology of AgNPs has been characterized. As seen in the TEM image in Fig. 7, the resultant AgNPs exhibit spherical nano-size. The histogram depicts the particle size distribution of the synthesized AgNPs@CCH with and without surfactant modification with a mean particle diameter of 11.07±1.39, 9.52±0.28, 8.87±0.14, and 8.38±0.11 for CCH, CCH-T, CCH-C, and CCH-R, respectively. The AgNPs synthesized in surfactant modified CCH shows the smaller size and narrower particle size distribution than in the unmodified CCH. This may be due to aggregates remains in existence in the AgNPs prepared in the CCH without surfactant inside. A noticeable result obtained in the CCH-R that shows a significantly high relative frequency in the 5 to 10 nm range indicates a more uniform particle size.

6. what is the state of silver nanoparticles in the black hydrogel?
Response: The state of AgNPs on the hydrogel can be observed from the TEM images, please refer to Figure 7.

7. Regarding the dosage of surfactants, why only choose 5mM and 10mM? I think it is necessary to explain.
The concentration of surfactant was chosen at higher than the CMC in order to create stable micelles for interact with AgNPs. Based on the cytotoxicity test, the surfactant concentration used in this study also did not show cytotoxic potential.

8. It is recommended to explain the interaction between AgNPs and hydrogel matrix, which is important for long-term antibacterial.
Response: We have added the explanation regarding the interaction between AgNPs and hydrogel matrix as suggested by the reviewer. Please refer to P20 L365-368 in the revised manuscript.
P20 L365-368: Once adsorbed, the Ag+ ions bind with the –OH groups in the CCH matrix and surfactant micelles. These bounded Ag+ was reduced by the NaBH4 that penetrates hydrogel then form AgNPs with retained interaction towards the -OH groups of CCH [65] and also with the surfactant in the modified CCH matrix

Reviewer #4:
Some minor queries / changes are suggested which could be examined by the editor(s) for the final decision.

· Authors should follow the proper journal format.

· Abstract: Rewrite the abstract.
Response: We have revised the format as suggested by the reviewer.

2. Materials and Methods Section:
Ø Mention the purity of the materials as well as the country name(s) for the all chemicals used in the studies. Authors should also mention the quality of water with its purity (sp conductivity).
Response: We have added the country name(s) for the all chemicals and the quality of water used in the studies as suggested by the reviewer. Please refer to Subsection 2.1 in the revised manuscript.
Ø Mention the experimental uncertainty.
Response: We have mention the uncertainty in the figure caption of the experimental data.

4. Conclusions Section:
Ø Rewrite the conclusions section.
Response: We have revised the conclusion section.

Reviewer #5:
The authors present an interesting study on increasing the loading of AgNP into hydrogels by using
Surfactants inside hydrogels prepared from cellulose carbamate (CC). The authors performed antibacterial studies on two bacterial strains (gram positive and negative) to test their prepared loaded hydrogels.
The language of the whole manuscript needs to be completely revised. The authors claim the preparation of AgNPs inside Cellulose hydrogels, however, in order to prepare AgNPs, some characterization are certainly needed (absorption spectrum to see the peak whether it is monodispersed or aggregated, particle size (mono or polydispersed, zeta potential, SEM images, TEM images). After successful preparation and formation of well controlled size and shape of the AgNPs, then, they can be imbedded inside the cellulose hydrogel matrix
Response: In this study, we adapted adsorption technique of embedding of AgNPs into the hydrogel in order to establish interaction between AgNPs and surface active sites of CCH so that the loaded AgNPs can be retained in the CCH and released at the targeted site. We have added the TEM images to characterize the AgNPs.

In order to avoid aggregation of AgNPs, several capping agents have been reported (polymers, surfactants, etc), so did the author not use any capping agents to control the size the minimize the aggregation of AgNPs? And they rather used surfactants with cellulose matrix
Response: In an effort to minimize the aggregation of AgNPs, in this study AgNPs is incorporated onto cellulose carbamate hydrogel (CCH) and its surfactant-modified since surfactant has been known to have role in improving the stability of AgNPs [The Journal of Physical Chemistry C, 112, 5825-5834; Journal of colloid and interface science, 268, 357-361].

Why is the transparency of the hydrogels a factor studied by the authors?
Response: Transparency of the hydrogels is to study the effect of urea dose on the dissolution and distribution of cellulose which can be observed from transparency of the resulting hydrogel. The explanation can be found in P12 L248-251 in the revised manuscript.
P12 L248-251: In the carbamation process, the level of urea dose affects the transparency of the hydrogel. A high dose of urea resulting in the hydrogel with high transparency, where the transparency order is mercerized filter paper (MFP) ≤ CC1 < CC3 < CC5 (most transparent).

The presence of AgNPs within the cellulose matrix with different surfactants will certainly affect the aggregation behavior of AgNPs causing possible aggregation. Loading AgNPs should have been measured by dialysis bags method followed by Entrapment efficiency (EE%), loading capacity (LC%) which the authors did not do! These methods would give a direct indication on how much of AgNPs was trapped or loaded into the CCH
Response: In this study, the loaded AgNPs was analyzed by the use aqua regia which able to completely dissolve the hydrogel thus the results from AAS measurement were the amount of the AgNPs loaded in the CCH and its surfactant-modified.

There is no significance values reported by the authors in their studies (p value)
Response: We have added the statistical significance of the AgNPs loading capacity (LC) as suggested by the reviewer. Please refer to Figure 6 in the revised manuscript

Major revision is needed.

Reviewer #6:
In this article, cellulose carbamate hydrogel hybridized with silver nanoparticles was prepared by reducing AgNO3 in situ using sodium borohydride. The effect of adding surfactants in the loading of AgNO3 was investigated. Antibacterial activity against gram-negative bacteria and gram-positive bacteria were also investigated.
The manuscript is within the scope of the journal. However, this manuscript could be accepted for publication after major revision. The detailed comments are listed as follows:
· Figure 1 needs revision: The repeating unit is wrongly depicted. Please show either the left (preferred) or the right oxygen atom of the glycosidic bond but not both of them. Also, the resolution needs modification.
Response: We have revised the Figure 1.

· The major issue of this paper is that the design of antibacterial hydrogel actually falls short of innovation.
Response: We thank the reviewer for the comments. In this study, we introduce a new approach in the loading of AgNPs that is by adsorption technique. We have emphasized the novelty of this study as suggested by the reviewer. Please refer to P5 L87-94 in the revised manuscript.
P5 L87-94: A new-facile approach in the incorporation of AgNPs to hydrogel was introduced. That is, by employing adsorption techniques of Ag+ ions onto the surface active sites of the hydrogel, followed by in situ reductions of the attached Ag+ ions. This approach allows the binding of large numbers of AgNP to the surface of the hydrogel dressing, thereby enhancing the antibacterial activity against the target bacteria. Furthermore, this approach could be an improvement over the conventional AgNPs hydrogel-dressing—wherein, in the preparation, AgNPs are integrated into the hydrogel matrix. So, the effectiveness of AgNPs in the center of the hydrogel matrix is reduced due to the difficulty of reaching the target bacteria.

· The authors should compare and contrast the results with other publications. A better job at this point must be required.
Response: We have added comparison study which highlighting the greater antimicrobial activity of the prepared hydrogel dressing compare to the reported hydrogel-dressings (P29 L-462-468).
P29 L-462-468: Comparative studies of the antimicrobial effects of AgNPs and AgNPs-hybrid materials are summarized in Table S2. As shown from the ZOI value, AgNPs@CCH-R (in this work) showed better antimicrobial activity compared to AgNPs-hybrid carboxymethyl cellulose hydrogels [79], PPEGMA-ran-PAA copolymer hydrogel [80], and bioreduced AgNPs [81]. However, the material prepared in this work has lower activity compared to AgNPs-hybrid polycaprolactone nanofibers [82]. Nevertheless, AgNPs@CCH, which are modified by surfactants, can easily and cheaply be prepared from sustainable raw materials.

· point out the limitations/shortcomings of this system.
Response: We have added the limitations/shortcomings of the system used in this study. Please refer to P29 L478-479 in the revised manuscript.
P29 L478-479: While these findings unlocked a new approach for AgNPs loading, a limitation may raise due to the variations of the adsorption capacity of each material.

· Please explain the mechanism of binding of AgNPs with hydrogel and the benefit of surfactants.
Response: We have added the explanation regarding the mechanism of binding of AgNPs with hydrogel and the benefit of surfactants as suggested by the reviewer. Please refer to P20 L365-368 and P22 L377-385 in the revised manuscript.
P20 L365-368: Once adsorbed, the Ag+ ions bind with the –OH groups in the CCH matrix and surfactant micelles. These bounded Ag+ was reduced by the NaBH4 that penetrates hydrogel then form AgNPs with retained interaction towards the -OH groups of CCH [65] and also with the surfactant in the modified CCH matrix.

P22 L377-385: The effect of surfactant on the morphology of AgNPs has been characterized. As seen in the TEM image in Fig. 7, the resultant AgNPs exhibit spherical nano-size. The histogram depicts the particle size distribution of the synthesized AgNPs@CCH with and without surfactant modification with a mean particle diameter of 11.07±1.39, 9.52±0.28, 8.87±0.14, and 8.38±0.11 for CCH, CCH-T, CCH-C, and CCH-R, respectively. The AgNPs synthesized in surfactant modified CCH shows the smaller size and narrower particle size distribution than in the unmodified CCH. This may be due to aggregates remains in existence in the AgNPs prepared in the CCH without surfactant inside. A noticeable result obtained in the CCH-R that shows a significantly high relative frequency in the 5 to 10 nm range indicates a more uniform particle size.

· The author should check the article carefully to avoid some grammatical mistakes.
Response: We thank the reviewer for this comment, we have carefully check the whole manuscript and revised the grammatical mistakes.

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Date: Sep 19 2020 12:29:10:577PM
To: "Shella Permatasari Santoso" shella_p5@yahoo.com
From: "Materials Science & Engineering C" msc@elsevier.com
Subject: Decision on submission to Materials Science & Engineering C

Manuscript Number: MSEC_2020_1651R1

Fabrication of cellulose carbamate hydrogel-dressing with rarasaponin surfactant for enhanching adsorption of silver nanoparticles and antibacterial activity

Dear Dr Santoso,

Thank you for submitting your manuscript to Materials Science & Engineering C.

I am pleased to inform you that your manuscript has been accepted for publication.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Materials Science & Engineering C and hope you will consider us again for future submissions.

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Maria Vamvakaki
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Materials Science & Engineering C

Fabrication of cellulose carbamate hydrogel-dressing with rarasaponin surfactant for enhancing adsorption of silver nanoparticles and antibacterial activity

--Manuscript Draft--

Manuscript Number:	MSEC_2020_1651R1
Article Type:	Research Paper
Keywords:	Cellulose carbamate; silver nanoparticles; wound dressing; antibacterial dressing
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	Aning Ayucitra
	Chintya Gunarto
	Yi-Hsu Ju
	Ming-Hua Ho
Abstract:	Bacterial contamination on external wounds is known to be a factor that prevents wound healing and triggers tissue damage. Hydrogel-dressings with antibacterial activity is a useful medical device to avoid this contamination, wherein the antibacterial activity can be provided via incorporation of silver nanoparticles (AgNPs). Contrary to the conventional two-step preparation of an AgNPs-loaded hydrogel (AgNPs@hydrogel), this work aims to establish a new and facile synthesis method employing the adsorption principle. Once AgNO 3 adsorbed into active sites of the hydrogels, in situ reductions using NaBH 4 was employed to produce AgNPs@hydrogel. The effect of surfactant addition on the AgNO 3 loading and the antibacterial activity of the resulting hydrogel dressing was investigated. The outcome of this work indicates that the addition of rarasaponin not only can increase the loading of AgNPs on cellulose carbamate hydrogel (CCH) but also significantly enhance the antibacterial activity of the resulted hydrogel-dressing. Superior to the other studied surfactant, the loading capacity (LC) of AgNPs is found to be 10.15, 9.94, and 7.53 mg/g for CCH modified with rarasaponin, CTAB, and Tween80, respectively. These findings conclude that the addition of surfactant, especially rarasaponin, can effectively improve the loading of AgNPs onto hydrogel-dressing via adsorption and promote the antibacterial activity. Furthermore, the cytotoxic test shows that the hydrogel-dressings have good biocompatibility toward skin fibroblast cells.
Suggested Reviewers:	Chun-Hui Zhou chc.zhou@aliyun.com
	Bo Feng b.feng@uq.edu.au
	Ianatul Khoiroh Ianatul.Khoiroh@nottingham.edu.my
Response to Reviewers:	Editor and Reviewer comments: Reviewer #1: In the present work, the authors reported an evolutionary progress in terms of strategies to increase the loading of AgNP into cellulose carbamate hydrogels by

utilizing surfactants. Overall, the article is well organized and its presentation is good. However, some minor issues still need to be improved:

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Response: We have added the explanation regarding the relationship between the Ag loading capacity and the antibacterial activity as suggested by the reviewer. Please refer to P26-27 L433-442 in the revised manuscript.
P26-27 L433-442: This released AgNP then damages bacterial cells by inducing the formation of hydroxyl radicals and penetrate into/interferes with DNA replication through the damaged cells, and thereby causing cell death. The more AgNPs come into contact, the damaging effect to the bacterial cells will be exaggerated. From the results of ZOI measurements, it was found that CCH-C had the highest antibacterial activity, followed by CCH-R > CCH-T > CCH. This is because the presence of surfactants helps increase the loading of AgNPs so that antimicrobial activity also increases. Distinct to other systems, in CCH-C–Since CTAB itself possess an antibacterial activity (as observed in negative control); thus the antibacterial activity does not solely depend on the amount of AgNPs in its matrix but also due to the presence of CTAB.

(2) The antibacterial activity of AgNPs@CCH is significant. From in vitro cell cultivation tests, AgNPs have been reported to be toxic potential to several human cell lines. Should biocompatibility and cytotoxicity be evaluated in this work?
Response: We have evaluated the cytotoxicity of the prepared hydrogels with AgNPs loading as suggested by the reviewer. Please refer to subsection 3.5 in P25 L414-419 in the revised manuscript.
P25 L414-419: The cytotoxicity evaluation is an extensive way of demonstrating the biocompatibility of the prepared hydrogel as a wound dressing material. Fig. 9 shows that none of the remaining cell viability was lower than 80%. According to ISO 10993-5: 1999, no cytotoxic potential in samples with cell viability higher than 70%. Therefore, it can be concluded that the AgNPs loaded hydrogels can maintain the normal function of skin fibroblasts. It can also be noted that the use of surfactant higher than CMC did not exhibit toxicity even in the high concentration of 10 mM.

(3) Elemental maps of the distribution of AgNPs is clear and important. The authors should provide elemental maps of Ag on CCH-T and CCH-C in Fig 6.
Response: We have provide the elemental maps of Ag on CCH-T and CCH-C as suggested by the reviewer. Please refer to Figure 6c in the revised manuscript.

(4) To clarify the antibacterial mechanism, the authors need to clarify how AgNPs disperse from hydrogels and come into contact with bacteria.
Response: We have added the explanation regarding the relationship between the Ag loading capacity and the antibacterial activity as suggested by the reviewer. Please refer to P26 L429-435 in the revised manuscript.
P26 L429-435: The antibacterial mechanism of AgNPs has been reported in many studies [69, 70]. Briefly, Ag, which is a soft acid, tends to interact with soft bases, here, sulfur contained in protein membranes and phosphorus in bacterial DNA. Since soft acid-soft base interaction is preferable than the interaction of AgNPs with the hydroxyl groups of cellulose hydrogel, thus AgNPs will be released from the hydrogel. This released AgNP then damages bacterial cells by inducing the formation of hydroxyl radicals and penetrate into/interferes with DNA replication through the damaged cells, and thereby causing cell death.

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Reviewer #2:
The manuscript reported the synthesis of cellulose carbamate (CC) loaded with silver nanoparticles (AgNPs) using sodium borohydride as a reducing agent. The authors further studied the effect of adding surfactants in promoting the loading of AgNPs. Overall, the authors adequately incorporated in this manuscript with all results in terms of preparation and characterization using various characterization techniques with sufficient explanations. Finally, the Antibacterial test results also confirmed the AgNPs@CCH (modified with surfactants) have better antibacterial activity towards the tested bacterial strain. Although many reports published on hydrogels loaded with AgNPs using various reducing agents such as sodium borohydrides, citric acid, hydrazine, natural extracts, and glycols, the paper is interesting to readers of MSEC. Before acceptance of publication, the authors need to be addressed the following comments.

(1) The authors have explained the effect of the surfactant can increase the loading of AgNPs in the CCH using NaBH4 reducing agent. Some papers have reported the effect of functional groups on the hydrogel network could enhance the AgNPs loading. Thus the authors should explain the importance of present work in the introduction part with the other published papers.
Response: We have added importance of present work in the introduction part as suggested by the reviewer. Please refer to P5 L87-94 in the revised manuscript.
P5 L87-94: A new-facile approach in the incorporation of AgNPs to hydrogel was introduced. That is, by employing adsorption techniques of Ag+ ions onto the surface active sites of the hydrogel, followed by in situ reductions of the attached Ag+ ions. This approach allows the binding of large numbers of AgNP to the surface of the hydrogel dressing, thereby enhancing the antibacterial activity against the target bacteria. Furthermore, this approach could be an improvement over the conventional AgNPs hydrogel-dressing–wherein, in the preparation, AgNPs are integrated into the hydrogel matrix. So, the effectiveness of AgNPs in the center of the hydrogel matrix is reduced due to the difficulty of reaching the target bacteria.

(2) In general, tween80, it can act as reducing and stabilizing agents for the production of metal salts to metal nanoparticles. Thus, the silver nanoparticles can form without using NaBH4, please justify. (doi: 10.1186/1556-276X-7-612 <https://dx.doi.org/10.1186%2F1556-276X-7-612>)
Response: Although Tween80 is reported to be oxidized in the presence of silver ions and act as reducing agent to synthesize AgNP, based on <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3503618/>
the reaction happens at 90˚C or higher temperature.
In this study, all reactions were done at room temperature thus Ag+ ions loaded with CCH-T were not reduced as indicated by the absence of hydrogel color changes, because the temperature has been known to play a role in the formation of AgNPs.

(3) In conclusion, the authors mentioned the developed AgNPs@CCH could be applied in wound dressing. However, toxicity is a major concern for particular biological applications. For this, the authors should incorporate cytotoxicity test results for all hydrogels, so that the conclusion well support with results.
Response: We have evaluated the cytotoxicity of the prepared hydrogels with AgNPs loading as suggested by the reviewer. Please refer to subsection 3.5 in P25 L414-419 in the revised manuscript.
P25 L414-419: The cytotoxicity evaluation is an extensive way of demonstrating the biocompatibility of the prepared hydrogel as a wound dressing material. Fig. 9 shows that none of the remaining cell viability was lower than 80%. According to ISO 10993-5: 1999, no cytotoxic potential in samples with cell viability higher than 70%. Therefore, it can be concluded that the AgNPs loaded hydrogels can maintain the normal function of skin fibroblasts. It can also be noted that the use of surfactant higher than CMC did not exhibit toxicity even in the high concentration of 10 mM.

(4) The citations were a little bit inadequate; I suggest some of the articles.
https://doi.org/10.1016/j.matlet.2016.08.043<https://doi.org/10.1016/j.matlet.2016.08.043> https://doi.org/10.1002/pi.4789<https://doi.org/10.1002/pi.4789>
https://doi.org/10.1002/jbm.a.34991 <https://doi.org/10.1002/jbm.a.34991>
Response: We have added some references as suggested by the reviewer.

Reviewer #3:
This work (MSEC_2020_1651) demonstrated the antibacterial activities of Ag/carbamate cellulose nanocomposite hydrogels prepared by using three different surfactants. CCH-R sample showed the highest Ag loading and best antibacterial property. How about improve the Ag loading by using higher concentration of AgNO3 solution?
Response: We thank the reviewer for the comments. In this work, the amount of AgNO3 added during the loading is already excessive which is 106.28 fold higher than the highest LC that can be achieved by CCH-T10. Therefore, the use of higher concentration would not contribute to the increase of LC.

What is the effect of surfactants for the distribution and size of Ag nanoparticles?
Response: In order to know the effect of surfactant on the distribution we provide the elemental maps of Ag on hydrogels in Figure 6 in the revised manuscript. The size of AgNPs were determined by using TEM analysis as shown in Figure 7 in the revised manuscript. The related discussion has been added in P22 L377-385.
P22 L377-385: The effect of surfactant on the morphology of AgNPs has been characterized. As seen in the TEM image in Fig. 7, the resultant AgNPs exhibit spherical nano-size. The histogram depicts the particle size distribution of the synthesized AgNPs@CCH with and without surfactant modification with a mean particle diameter of 11.07±1.39, 9.52±0.28, 8.87±0.14, and 8.38±0.11 for CCH, CCH-T, CCH-C, and CCH-R, respectively. The AgNPs synthesized in surfactant modified CCH shows the smaller size and narrower particle size distribution than in the unmodified CCH. This may be due to aggregates remains in existence in the AgNPs prepared in the CCH without surfactant inside. A noticeable result obtained in the CCH-R that shows a significantly high relative frequency in the 5 to 10 nm range indicates a more uniform particle size.

What is the potential application of this material? wound dressing? Therefore, some issues should be responded before publication.
Response: The studied materials are potential as wound dressing, it has good antibacterial activities towards E. coli and S. aureus. Also, based on cytotoxic study, we have demonstrated the non-toxic behavior of the materials to the normal cells.

Specific comments
1. Some related references about cellulose-based hydrogels with antibacterial activity should be included in the introduction of the manuscript, such Carbohydrate Polymers2016, 137, 59
Response: We have added some related references regarding the cellulose-based hydrogels with antibacterial activity in the introduction as suggested by the reviewer. Please refer to P4 L67-68 in the revised manuscript.

2. Author are suggested to show the advantage of carbamate cellulose used here in comparison with other cellulose derivatives for the fabrication of cellulose-based hydrogels.
Response: We have added the superiority of cellulose carbamate as suggested by the reviewer. Please refer to P4-5 L77-86 in the revised manuscript.
P4-5 L77-86: Various dissolution methods have been established to resolve this limitation, such as the viscose process, carbamation, lyocell process, and the use of the ionic-liquid system [37]. Carbamation of cellulose to produce cellulose derivatives (i.e., cellulose carbamate) is known as an environmental dissolution method of cellulose compared to another soluble cellulose derivative (i.e., cellulose xanthogenate) which the synthesis accompanied by the generation of hazardous byproducts. Cellulose carbamate (CC) has better solubility in alkaline and organic solution compared to cellulose [38]. Furthermore, CC reported to possess bacteriostatic properties, which make it more resistant against microbial contamination and enzymatic-cleavage compare to cellulose. Gan et al. also reported that CC-based hydrogel has an enhanced porosity compare to cellulose hydrogels [39], and therefore, in this work, the carbamation process was chosen to dissolve the cellulose.

3. What is the degree of substitution of carbamate cellulose?
Response: We have added the degree of substitution (i.e., 0.34±0.03) of the synthesized cellulose carbamate as inquired by the reviewer. Please refer to P12 L257-259 in the revised manuscript.
P12 L257-259: From the results, it was found that CC5, with the ratio of cellulose to urea 1: 5, has the best transparency with a degree of substitution of 0.34±0.03 as calculated using the published equation [48] so that this ratio was chosen for further studies.

4. For the formation of hydrogels and the crosslinking of cellulose derivatives, some classic references should be cited instead of [48], such Macromolecular Bioscience2007,7, 804; European Polymer Journal2010, 46, 92; Macromolecules2011, 44, 1642.
Response: We have added some references regarding the formation of hydrogels and the crosslinking of cellulose derivatives as suggested by the reviewer.

5. TEM is a useful method to investigate the size and distribution of silver nanoparticles in the hydrogel matrix, which will be benefit to understand the role of surfactants.
Response: We have added the TEM results to better understand the role of surfactant to the size of AgNPs. Please refer to Figure 7 and discussion P22 L377-385 in the revised manuscript.
P22 L377-385: The effect of surfactant on the morphology of AgNPs has been characterized. As seen in the TEM image in Fig. 7, the resultant AgNPs exhibit spherical nano-size. The histogram depicts the particle size distribution of the synthesized AgNPs@CCH with and without surfactant modification with a mean particle diameter of 11.07±1.39, 9.52±0.28, 8.87±0.14, and 8.38±0.11 for CCH, CCH-T, CCH-C, and CCH-R, respectively. The AgNPs synthesized in surfactant modified CCH shows the smaller size and narrower particle size distribution than in the unmodified CCH. This may be due to aggregates remains in existence in the AgNPs prepared in the CCH without surfactant inside. A noticeable result obtained in the CCH-R that shows a significantly high relative frequency in the 5 to 10 nm range indicates a more uniform particle size.

6. what is the state of silver nanoparticles in the black hydrogel?
Response: The state of AgNPs on the hydrogel can be observed from the TEM images, please refer to Figure 7.

7. Regarding the dosage of surfactants, why only choose 5mM and 10mM? I think it is necessary to explain.
The concentration of surfactant was chosen at higher than the CMC in order to create stable micelles for interact with AgNPs. Based on the cytotoxicity test, the surfactant concentration used in this study also did not show cytotoxic potential.

8. It is recommended to explain the interaction between AgNPs and hydrogel matrix, which is important for long-term antibacterial.
Response: We have added the explanation regarding the interaction between AgNPs and hydrogel matrix as suggested by the reviewer. Please refer to P20 L365-368 in the revised manuscript.
P20 L365-368: Once adsorbed, the Ag+ ions bind with the –OH groups in the CCH matrix and surfactant micelles. These bounded Ag+ was reduced by the NaBH4 that penetrates hydrogel then form AgNPs with retained interaction towards the -OH groups of CCH [65] and also with the surfactant in the modified CCH matrix

Reviewer #4:
Some minor queries / changes are suggested which could be examined by the editor(s) for the final decision.

·Authors should follow the proper journal format.
·Abstract: Rewrite the abstract.
Response: We have revised the format as suggested by the reviewer.

2.Materials and Methods Section:

Ø Mention the purity of the materials as well as the country name(s) for the all chemicals used in the studies. Authors should also mention the quality of water with its purity (sp conductivity).
Response: We have added the country name(s) for the all chemicals and the quality of water used in the studies as suggested by the reviewer. Please refer to Subsection 2.1 in the revised manuscript.
Ø Mention the experimental uncertainty.
Response: We have mention the uncertainty in the figure caption of the experimental data.

4. Conclusions Section:
ØRewrite the conclusions section.
Response: We have revised the conclusion section.

Reviewer #5:
The authors present an interesting study on increasing the loading of AgNP into hydrogels by using
Surfactants inside hydrogels prepared from cellulose carbamate (CC). The authors performed antibacterial studies on two bacterial strains (gram positive and negative) to test their prepared loaded hydrogels.
The language of the whole manuscript needs to be completely revised. The authors claim the preparation of AgNPs inside Cellulose hydrogels, however, in order to prepare AgNPs, some characterization are certainly needed (absorption spectrum to see the peak whether it is monodispersed or aggregated, particle size (mono or polydispersed, zeta potential, SEM images, TEM images). After successful preparation and formation of well controlled size and shape of the AgNPs, then, they can be imbedded inside the cellulose hydrogel matrix
Response: In this study, we adapted adsorption technique of embedding of AgNPs into the hydrogel in order to establish interaction between AgNPs and surface active sites of CCH so that the loaded AgNPs can be retained in the CCH and released at the targeted site. We have added the TEM images to characterize the AgNPs.

In order to avoid aggregation of AgNPs, several capping agents have been reported (polymers, surfactants, etc), so did the author not use any capping agents to control the size the minimize the aggregation of AgNPs? And they rather used surfactants with cellulose matrix
Response: In an effort to minimize the aggregation of AgNPs, in this study AgNPs is incorporated onto cellulose carbamate hydrogel (CCH) and its surfactant-modified since surfactant has been known to have role in improving the stability of AgNPs [The Journal of Physical Chemistry C, 112, 5825-5834; Journal of colloid and interface science, 268, 357-361].

Why is the transparency of the hydrogels a factor studied by the authors?
Response: Transparency of the hydrogels is to study the effect of urea dose on the dissolution and distribution of cellulose which can be observed from transparency of the resulting hydrogel. The explanation can be found in P12 L248-251 in the revised manuscript.
P12 L248-251: In the carbamation process, the level of urea dose affects the transparency of the hydrogel. A high dose of urea resulting in the hydrogel with high transparency, where the transparency order is mercerized filter paper (MFP) ≤ CC1 < CC3 < CC5 (most transparent).

The presence of AgNPs within the cellulose matrix with different surfactants will certainly affect the aggregation behavior of AgNPs causing possible aggregation. Loading AgNPs should have been measured by dialysis bags method followed by Entrapment efficiency (EE%), loading capacity (LC%) which the authors did not do! These methods would give a direct indication on how much of AgNPs was trapped or loaded into the CCH
Response: In this study, the loaded AgNPs was analyzed by the use aqua regia which able to completely dissolve the hydrogel thus the results from AAS measurement were the amount of the AgNPs loaded in the CCH and its surfactant-modified.

There is no significance values reported by the authors in their studies (p value)

Response: We have added the statistical significance of the AgNPs loading capacity (LC) as suggested by the reviewer. Please refer to Figure 6 in the revised manuscript

Major revision is needed.

Reviewer #6:
In this article, cellulose carbamate hydrogel hybridized with silver nanoparticles was prepared by reducing AgNO3 in situ using sodium borohydride. The effect of adding surfactants in the loading of AgNO3 was investigated. Antibacterial activity against gram-negative bacteria and gram-positive bacteria were also investigated.
The manuscript is within the scope of the journal. However, this manuscript could be accepted for publication after major revision. The detailed comments are listed as follows:
·Figure 1 needs revision: The repeating unit is wrongly depicted. Please show either the left (preferred) or the right oxygen atom of the glycosidic bond but not both of them. Also, the resolution needs modification.
Response: We have revised the Figure 1.

·The major issue of this paper is that the design of antibacterial hydrogel actually falls short of innovation.
Response: We thank the reviewer for the comments. In this study, we introduce a new approach in the loading of AgNPs that is by adsorption technique. We have emphasized the novelty of this study as suggested by the reviewer. Please refer to P5 L87-94 in the revised manuscript.
P5 L87-94: A new-facile approach in the incorporation of AgNPs to hydrogel was introduced. That is, by employing adsorption techniques of Ag+ ions onto the surface active sites of the hydrogel, followed by in situ reductions of the attached Ag+ ions. This approach allows the binding of large numbers of AgNP to the surface of the hydrogel dressing, thereby enhancing the antibacterial activity against the target bacteria. Furthermore, this approach could be an improvement over the conventional AgNPs hydrogel-dressing–wherein, in the preparation, AgNPs are integrated into the hydrogel matrix. So, the effectiveness of AgNPs in the center of the hydrogel matrix is reduced due to the difficulty of reaching the target bacteria.

· The authors should compare and contrast the results with other publications. A better job at this point must be required.
Response: We have added comparison study which highlighting the greater antimicrobial activity of the prepared hydrogel dressing compare to the reported hydrogel-dressings (P29 L-462-468).
P29 L-462-468: Comparative studies of the antimicrobial effects of AgNPs and AgNPs-hybrid materials are summarized in Table S2. As shown from the ZOI value, AgNPs@CCH-R (in this work) showed better antimicrobial activity compared to AgNPs-hybrid carboxymethyl cellulose hydrogels [79], PPEGMA-ran-PAA copolymer hydrogel [80], and bioreduced AgNPs [81]. However, the material prepared in this work has lower activity compared to AgNPs-hybrid polycaprolactone nanofibers [82]. Nevertheless, AgNPs@CCH, which are modified by surfactants, can easily and cheaply be prepared from sustainable raw materials.

· point out the limitations/shortcomings of this system.
Response: We have added the limitations/shortcomings of the system used in this study. Please refer to P29 L478-479 in the revised manuscript.
P29 L478-479: While these findings unlocked a new approach for AgNPs loading, a limitation may raise due to the variations of the adsorption capacity of each material.

·Please explain the mechanism of binding of AgNPs with hydrogel and the benefit of surfactants.
Response: We have added the explanation regarding the mechanism of binding of AgNPs with hydrogel and the benefit of surfactants as suggested by the reviewer. Please refer to P20 L365-368 and P22 L377-385 in the revised manuscript.
P20 L365-368: Once adsorbed, the Ag+ ions bind with the –OH groups in the CCH matrix and surfactant micelles. These bounded Ag+ was reduced by the NaBH4 that penetrates hydrogel then form AgNPs with retained interaction towards the -OH groups

	of CCH [65] and also with the surfactant in the modified CCH matrix. P22 L377-385: The effect of surfactant on the morphology of AgNPs has been characterized. As seen in the TEM image in Fig. 7, the resultant AgNPs exhibit spherical nano-size. The histogram depicts the particle size distribution of the synthesized AgNPs@CCH with and without surfactant modification with a mean particle diameter of 11.07±1.39, 9.52±0.28, 8.87±0.14, and 8.38±0.11 for CCH, CCH-T, CCH-C, and CCH-R, respectively. The AgNPs synthesized in surfactant modified CCH shows the smaller size and narrower particle size distribution than in the unmodified CCH. This may be due to aggregates remains in existence in the AgNPs prepared in the CCH without surfactant inside. A noticeable result obtained in the CCH-R that shows a significantly high relative frequency in the 5 to 10 nm range indicates a more uniform particle size. · The author should check the article carefully to avoid some grammatical mistakes. Response: We thank the reviewer for this comment, we have carefully check the whole manuscript and revised the grammatical mistakes.