Hyaluronic Acid (HA)-Methylcellulose (MC)-Based Hydrogel with Antibacterial AgNPs as a Post-Surgery Intraperitoneal Anti-Adhesive Physical Barrier

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Abstract

Post-surgery Peritoneal adhesion is a common problem causing complication after abdominal surgery, even death. This study aims to prove the hydrogel potential and obtain the optimum composition of AgNPs as intraperitoneal anti-adhesive physical barrier. The synthesize started with mixture of HA-MC from freeze-dry process with variation of AgNPs of 0, 1, 2, 3, and 5 ppm in Phosphate Buffer Saline (PBS). The result of Fourier Transform Infrared (FTIR) showed the crosslinking bonding in samples showed by the new imine bond on 1556.55 cm^{-1} and 1548.84 cm^{-1} on 0 and 3 ppm of AgNPs concentration. The composition of optimum AgNPs as HA-MC-AgNPs hydrogel composition was the sample with 3 ppm with degradation rate of 90% on the 11th day which is approaching the ideal value of intraperitoneal antiadhesive physical barrier degradation rate and the cell viability of 59.45% which showed a nontoxic property. It also has high sensitivity to bacteria with clear zone diameter of 23.5 mm and swelling ratio which meets the standard and good mechanical properties. The HA-MC-AgNPs composite is safe and has potential to be applied as intraperitoneal antiadhesive physical barrier hydrogel based on the functional groups, degradation rate, cell viability, antibacterial test, mechanical test and swelling test.

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Introduction

Intraperitoneal adhesion is a common problem that often occurs on post-surgery which causes severe surgical complication. The surgical procedure is one of the adhesion cause. The intraperitoneal adhesion is defined as abnormal fibroses band occurring on the abdominal cavity and organs surface which was reported more than 90% of hernia surgery cases.[1] The prevalence of intraabdominal adhesion is around 67-93% after laparotomic surgery and reaches 97% on gynecological surgery. The adhesion of wound and omentum was occurred on 80% of patients and around 50% involving the intestines.

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The adhesion causing the mechanical intestine obstruction occurs on small intestines of 65-75%, infertility of 15-20% and chronic pain in pelvic area of 20-50% and more than 342000 actions was performed to detach the adhesion in the U.S. in 2004 with high cost.[2]

According to Rink J and Ali A,[3] the incidence of obstruction caused by peritoneal adhesion was on the second or third position after hernia inguinalis and colon malignancy. The intraperitoneal adhesion is the most common cause of obstructive ileum. In Indonesia, the case of inpatient paralytic obstructive ileum and obstruction without hernia has been recorded in the amount of 7059 cases and outpatient of that was 7024 in 2004.[3] Around 50-70% from all cases, the adhesion could be caused by intraperitoneal surgery history or intraabdominal inflammation process. More than 34% of the adhesion was re-hospitalized due to complication regarding adhesion, with the mortality number of 4.6-13%.[4]

The intraperitoneal adhesion is a common case encountered by the surgeons after abdominal surgery. Based on its etymology, the intraperitoneal adhesion could be classified as a default or possessed as a post-inflammation reaction or post-surgery as the highest number of cases. The post-surgical adhesion could cause intestine obstruction, chronic stomachache, infertility of female and the difficulty in re-surgery. The adhesion formation of adhesion is still the main cause of ileum obstruction.[5] The prevention of the intraperitoneal adhesion is the goal in the surgical practice and there are many strategies have been carried out but there was no perfect solution. There are some local or systemic pharmacological agents such as anti-inflammation drugs, anti-coagulant, including aspirin, dexamethasone, and heparin, that have been evaluated to prevent post-surgery adhesion. But, none of them could bring the satisfying result. The liquid barrier such as crystalloid (NaCl and ringer lactate), and polymeric solution (N, O-carboxylmethyl chitosan (NOCC), sodium hyaluronate, and carboxylmethyl cellulose (CMC)) was easily absorbed even using in high amount.[6]

To overcome the lack in the intraperitoneal adhesion case, there were some tests concerning on some barriers which were performed to prevent the adhesion such as film, membrane, and hydrogel which based on natural or synthetic biomaterials. However, film and membrane have a practical difficulty due to its placement in the surgical sites and could not prevent the adhesion and cause the complication.[7] In recent years, hydrogel was preferred due to its easy application. Hydrogel has a potential to mimic the extracellular matrix which has function as a tissue regeneration agent for wound healing. Hydrogel has some advantages, such as covering the wound area and all organ surface with its complex geometry when injecting it to the body so that it could be the physical barrier or direct contact with abdomen wall or among the organ surfaces.[8] In the study of Li et al. (2014), the ideal anti-adhesion material has to meet the following conditions, such as the convenience in application and handling, non-toxic, biocompatible, the ability to cover the wound with its complex geometry and could stay for 5-7 days, biodegradable with degradation time of exactly 7 days.[9]

In the study of Ito et al. (2007), the physical barrier was synthesized as a hydrogel from hyaluronic acid and cellulose derivatives.[10] The compound of hyaluronic acid-methylcellulose was the most effective in preventing the intraperitoneal adhesion and already went through in vivo test in rabbit's abdomen wall which was found that there was no adhesion after a week of surgery. But, in the previous study, there was no synthesize with antibacterial addition while the infection prevalence of bacteria in the nonsterile abdomen cavity is high. In Asia, the infection prevalence of *Staphylococcus aureus Resisten Metisilin* (MRSA) now reached 70%, and in Indonesia, in 2006, its prevalence was 23.5%. It has been reported that the nosocomial infection caused 88000 patients in the world died every year.[11] Thus, this study will focus on the synthesize of physical barrier in the form of hydrogel based on hyaluronic acid-methylcellulose with the addition of antibacterial nano silvers (AgNPs). AgNPs was already used in medical application and can increase the wound healing.[12] In the study of Nanda and Saravanan (2009), AgNPs as antibacterial agent could inhibit the growth of bakteri *Staphylococcus aureus*and *Streptococcus pyogenes* which are ones of the main bacteria causing post-surgery wound infection (ILO).[13] Methylcellulose (MC) has good biocompatibility and can increase the mechanical properties of the hydrogel.[14] Hydrogel with hyaluronic acid as its main component is biocompatible, non-immunogenic, non-toxic, and naturally bio absorbable. Hyaluronic acid also could increase the proliferation on the mesothelial peritoneal cells.[15] The characterization that were performed to observe the potential of hyaluronic acid - methylcellulose-based hydrogel with antibacterial agent AgNPs in this study was functional group test, swelling test, degradation test, cytotoxicity test, and antibacterial test.

Materials and methods

The research took place in Institute Tropical Disease (ITD), Universitas Airlangga for synthesizing the hydrogels, degradation test, swelling test and antibacterial test, Pusvetma Laboratory Surabaya for cytotoxicity test, Integrated Laboratory in Faculty of Mathematics and Natural Science, State University of Surabaya for freeze drying process, and chemistry laboratory in Science and Technology Faculty, Universitas Airlangga for functional groups test.

Hyaluronic acid (Mw 490 kDa), methylcellulose, ethylene glycol, adipic dihydrazide (ADH), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC), hydroxybenzotriazole (HOBt), sodium periodate ($NaIO_4$), Phospate Body Simulation (PBS) solution, $AgNO_3$, trisodium sitrat, aquades, dimethylsulfoxide (DMSO), NaOH, NaCl, HCl, ethanol.

To form hydrogel, all of the materials, such as hyaluronic acid, methylcellulose and nano silver were mixed in the PBS solution.

The hyaluronic acid was modified with adipic di-hydrazide acid (ADH). 1 gr of HA was dissolved in distilled water to form 1 mg/ml HA. 3.5 gr of ADH was added to the solution and mixed for 30 minutes until the pH becomes 6.8 with the addition of 0.1 M NaOH and 0.1 M HCl. 0.78 gr EDC and 0.77 gr HOBt were dissolved in DMSO/H_2O (1:1 v/v) 5 ml each and were added to the mixture of HA and ADH. The pH of the solution was adjusted to 6.8 with the addition of 0.1 N HCl for 4 hours minimum. The reaction was left for a night, then the pH was adjusted to 7.0. the solution of HA-ADH then was deposited in ethanol. The precipitate was dissolved again in aquades and freeze-dried and saved at temperature of 4 °C.[16]

The methylcellulose was prepared with periodate oxidation. 1.5 gr Methylcellulose was dissolved in 150 ml aquades, then 802 mg sodium periodate was added and mixed for 2 hours. 0.2 ml ethylene glycol was added to stop the reaction, then the MC-aldehyde was freeze-dried and saved at temperature of 4 °C.[10]

The synthesize of AgNPs was performed with chemical reduction method. $AgNO_3$ and trisodium citrate was prepared with dissolving them in the aquades (10^{-4} M $AgNO_3$ solution: trisodium citrate solution 10^{-3}M =10:1).

The $AgNO_3$ solution was boiled on 300-330 °C using a stirrer for 15 minutes. The trisodium citrate was added to the $AgNO_3$ solution. After 30 minutes, the temperature was lowered to 250 °C. At the minute of 32 and 42, the temperature was lowered to 250 °C and 100 °C respectively. The stirrer speed was ±5 rpm until the suspension becomes yellow which indicates the formation of AgNPs.[17]

Results

The hydrogel was synthesized andvisually, the sample with the addition of AgNPs solution becomes yellow due to the golden color of AgNPs shoen in Figure 1.

Swelling test

The hydrogels were freeze-dried. The weight of before and after immersion in PBS for 24 hours were performed to observe the ability of the hydrogel in absorbing the PBS solution. The result was shown in Figure 2. The swelling ratio for sample 0 ppm, 1 ppm, 2 ppm, 3 ppm, and 5 ppm are 215.7%,198.5%,184.6%,175.8% and 153.2%.

Degradation test

The degradation test of the hydrogels in PBS were performed for 1, 3, 5, 7, 9, 11 days to observe the hydrogel degradation in PBS solution in physiological pH with normal body temperature of 37 °C. The mass loss percentage was obtained from this test for sample 0 ppm, 1 ppm, 2 ppm, 3 ppm, and 5 ppm are 95.2%, 93.2%, 90.2%, 90%, and 87.8% respectively on the 11^{th} day.

Cytotoxicity test

The cytotoxicity test was performed with ELISA reader and BHK-21 Cell. Figure 3 showed that when the concentration of AgNPs was increasing, the cell viability was decreasing because the released AgNPs to the cells. The cell viability for sample 0 ppm, 1 ppm, 2 ppm, 3 ppm, and 5 ppm are 64.10%,62.87%,61.82%,59.45% and 58.53%.

Functional group test

The functional group characterization was performed after the synthesize of the hydrogels was done with FTIR spectroscopy in range of 400-4000 cm^{-1}. In this test, the sample with 0 and 3 ppm of AgNPs were chosen to observe the polymerization reaction between HA-ADH and MC-Ald. The result was shown in Table 1.

Antibacterial test

Antibacterial test was performed to observe the resistance of hydrogel sample to bacteria with gram positive bacteria, which was *Staphylococcus Aureus*in order to obtain the anti-microbial activity on the sample. The result was shown in Table 2.

Tensile Strength Test

The tensile strength test is a parameter to evaluate the mechanical properties from a material. The tensile strength was performed on the HA-MC-AgNPs hydrogels. Tensile strength and elongation at break are important physical factors of hydrogels that indicate their flexibility as they are applied as physical barrier in the abdomen area because when the materials are too stiff, they will harm the internal organ of abdomen. Based on Figure 5, the variation of AgNPs was not affecting the tensile strength of the hydrogels. For the elongation at break, the higher the concentration of AgNPs, the lower the elongation at break, but the difference is still not significant.

Discussion

The HA-MC-AgNPs hydrogels have high potential as antiadhesive barrier from its physical characteristics, such as the swelling test to observe the absorption level of the hydrogels towards liquid, the degradation test to observe the mass loss from the hydrogels, the tensile strength test to observe the elasticity of the hydrogels and antibacterial test to observe the antimicrobial activity of AgNPs.

Based on Figure 2. The swelling ratio was decreasing when the concentration of AgNPs increased.The swelling ration value of hydrogel for preventive application to intraperitoneal adhesion in the abdomen is 123-225%.[10] This occurred due to the crosslinking of silver oxide nanoparticles with electron rich O and N atom from hydroxyl and amine group in the crosslinking of hyaluronic acid and methylcellulose. Moreover, all the samples still met the swelling ratio standard for preventive application for intraperitoneal adhesion.

Based on Figure 2. The degradation rate was decreasing when the concentration of AgNPs increased. The level of degradation rate could be affected by the amount of crosslinking bond formed in the hydrogels.In this study, the polymerization type was chemical crosslinking between aldehyde group and di-hydrazide which formed hydrazine bond. The aldehyde group was obtained from methylcellulose and di-hydrazide was obtained from hyaluronic acid coupled with di-hydrazide group. The amount of formed aldehyde and di-hydrazide groups determine the amount of the crosslinking bond formed in the hydrogels. With high amount of crosslinking bond, the hydrogels would be more stable and would not degrade easily. The crosslinking bond creates the good mechanical stability and avoid the hydrophilic polymer chains dispersion to liquid phase.[18]

In the study of Ito et al. (2010), the hydrogel of hyaluronic acid and methylcellulose was degraded less than 80% on the 14th day and the in vivo test showed there was no adhesion score happened and indicated safe and had a potential to be post-surgery intraperitoneal adhesive physical barrier.[10] So that, in this study, all of the samples that were degraded around 87.8-95.2% on the 11th day could have the same potential to be intraperitoneal anti-adhesive agent in which still in the safe level, not exceeding 14 days.

Based on the Figure 3 showed that when the concentration of AgNPs was increasing, the cell viability was decreasing because the released AgNPs to the cells. The presence of AgNPs will damage the chemical structure which has Sulfur of Phosphor-based functional group such as DNA chain and inhibit DNA growth so that the cell growth was inhibited.[19] Besides that, AgNPs could interact to damage DNA, change the protein and enzyme properties which produce free radical and end up in cell lysis. AgNPs could be excreted from the body by excretion system if its function was done.[20]

Based on the Table 2. It was shown that the highest the AgNPs concentration, the bigger the formed clear zone which means that the sensitivity is increasing to the bacteria. If the sensitivity is increasing to the bacteria, then the sample is better to be applied as an antimicrobial agent based on the study of Mutia et al.[21]

Based on the functional group analysis on hydrogel IR spectra, the new peak was present on 1556.55 cm^{-1} dan 1548.84 cm^{-1}in Figure 4. It indicated that there was a new group of C=N or imine and suitable with the study of Wen Y.S. et al. (2010) where the polymerization can be marked with new bond in 1584 cm^{-1} corresponding to the functional group of HA-ADH and MC-Ald. At the same time, the stretched peak of C=O aldehyde at 1720-1740 cm^{-1} was gone due to the aldehyde consumption for forming imine bond between MC-Ald and HA-ADH.[22] The aldehyde group formed from oxidation of methylcellulose could be bonded with di-hydrazide on HA-ADH forming covalent bond of hydrazine which can form crosslinking and polymerization in the hydrogel.

Based on Figure 5, the variation of AgNPs was not affecting the tensile strength of the hydrogels because the concentration of AgNPs was so small and the difference in the concentration was not significant, which were from 0, 1, 2, 3, 4, and 5 ppm. Because of the small difference in the AgNPs, it will not affect the amount of force needed to tear the sample. For the elongation at break, the higher the concentration of AgNPs, the lower the elongation at break, but the difference is still not significant. It happened because the AgNPs could increase the material density in which AgNPs would be trapped in the hydrogel matrix.[23] The maximum force given by the abdomen wall in its elasticity limit without damaging is 220 KPa with strain of 0.15 and the maximum force limit that can break the abdomen wall is 560 KPa with strain of 1.[24] In this study, the maximum force give to all sample were 500 KPa in which was still in the maximum force limit that can break the abdomen wall. Unfortunately, the Young's modulus data could not be obtained from this test since the obtained data were not stress-strain graph so that the elastic region of the samples was not available.

Conclusions

The hyaluronic acid (HA)-Methylcellulose (MC) with the addition of antibacterial AgNPs in this study has a potential to be applied as an antiadhesive agent based on the functional group test (FTIR) which showed a crosslinking on the sample shown by the presence of new peak of imine group C=N at 1556.55cm^{-1} dan 1548.84cm^{-1} on AgNPs concentration of 0 and 3 ppm. The optimum AgNPs concentration as HA-MC-AgNPs hydrogel composite is sample with AgNPs concentration of 3 ppm with degradation rate of 90% at 11th day approaching the ideal value to be an intraperitoneal anti adhesive physical barrier. The cell viability test could reach 59.45% which is non-toxic and very sensitive to the bacteria based on the antibacterial test with clear zone diameter of 23.5 mm. The swelling ratio met the standard and the tensile strength was also good. The HA-MC-AgNPs composite is safe and has a potential to be applied as hydrogel for post-surgery intraperitoneal anti adhesive physical barrier based on functional groups, degradation rate, cytotoxicity, antibacterial test, tensile strength test and swelling test.

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Declaration of Interest

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References

1. Bolgen N, Vargel I, Korkusuz P, Menceloglu YZ, Piskin E. In vivo performance of antibiotic embedded electrospun PCL membranes for prevention of abdominal adhesions. J Biomed Mater Res, Part B. 2007;81(2):530-43.
2. Ward B.Research review: Abdominal adhesions: Current and novel therapies. J Surg Res. 2011;165:91-111.
3. Rink J, Ali A. Intestinal Obstruction After Totally Extraperitoneal Laparoscopic Inguinal Hernia Repair. Journal of Society of Laparoendoscopic Surgeons. 2004;8:89-92.
4. Hellebrekers BW, Kooistra T. Pathogenesis of Postoperative Adhesion Formation. Br J Surg. 2011;98(11):1503-1516.
5. Primariawan, Relly Y. Penggunaan Barrier adhesi pada Operasi Laparoskopi. Sci J Pharm Dev Med Appl. 2010;2 (3):3-8.
6. Zhang QY, Ma S, Xi D, Zhang WT, li AW. Administration of novel penicillamine-bound membrane: a preventive and therapeutic treatment for abdominal adhesions. BMC Surgery. 2011;11:5.
7. Chang KC, Tang TP, Hsu CK, Soung HS, Wang MH. Methyl Cellulose Enhance Gelatin Membrane as Guidance Channels For Peripheral Nerve Regeneration. Biomed Eng Appl Basis Commun. 2012;24(1):85-98.
8. Balakrishnan B, Mohanty M, Umashankar PR, Jayakrishnan A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials. 2005; 26:6335-42.
9. Li L, Wang N, Jin X, Deng R, Nie S, Sun L, Wu Q, Wei Y, Gong C. Biodegradable and injectable in situ cross-linking chitosanhyaluronic acid base hydrogel for postoperative adhesion prevention. Biomaterials. 2014;35: 3903-3917.
10. Ito T, Yeo Y, Highley CB, Bellas E, Benitez CA, Kohane DS. The prevention of peritoneal adhesions by in situ cross-linking hydrogels of hyaluronic acid and cellulose derivatives. Biomaterials. 2007;28:975-83.

11. Wahid MH. MRSA Update: Diagnosis dan tatalaksana.4th Symposium ofIndonesia Antimicrobial Resistence Watch (IARW). Dalam: Andra. Jakarta, 29Juni-1 Juli. Jakarta: Farmacia. 2007;64.
12. Tian J, Wong KKY, Ho CM, Lok CN, Yu WY, Che CM, Chiu JF, Tam PKH. Topical delivery of silver nano particles promotes wound healing. Chem Med Chem. 2007;2:129-36.
13. Nanda A, Saravanan M. Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. J Nanotechnol Biol Med. 2009; 5;452-56.
14. Liu W, Zhang B, Lu WW, Li X, Zhu D, Yao KD, Wang Q, Zhao C, Wang CA. Rapid temperature-responsive sol–gel reversible poly(N-isopropylacrylamide)-g-methylcellulose copolymer hydrogel. Biomaterials. 2004; 25: 3005-12.
15. Reijnen MM, Falk P, van Goor H, Holmdahl L. The antiadhesive agent sodium hyaluronate increases the proliferation rate of human peritoneal mesothelial cells. Fertil Steril. 2000; 74:146-51.
16. Jia XQ, Colombo G, Padera R, Langer R, Kohane DS. Prolongation of sciatic nerve blockade by in situ cross-linked hyaluronic acid. Biomaterials. 2004;25(19):4797-804.
17. Suriati G, Mariatti M, Azizan A. Synthesis of Silver Nanoparticles by Chemical reduction Method: Effect of Reducing Agent and Surfactant Concentration. Int J Automot Mech Eng. 2014;10:1920-1927.
18. Hennink WE, Nostrum CFV. Novel crosslinking methods to design hydrogels. Adv Drug Deliver Rev. 2002;54:13-36.
19. Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ. Interaction of silver nanoparticles with HIV. J Nanobiotechnol. 2005;3(1):6.
20. Hsin YH, Chen CF, Huang S, et al. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol. Lett. 2008;179:130-139
21. Mutia T, Eriningsih R, Safitri R. Membran Alginat Sebagai Pembalut Luka Primer dan Media Penyampaian Obat Topikal Untuk Luka yang Terinfeksi. Jurnal Riset Industri. 2011;5(2):161-174.
22. Wen YS, Yu CC, Feng HL . Injectable oxidized hyaluronic acid/adipic acid dihydrazide hydrogel for nucleus pulposus regeneration. Acta Biomaterialia 6. 2010;3044-3055.
23. Xiaodong W, Zhiming MW. High-Efficiency Solar Cells: Physics, Materials, and Devices. Springer Science & Business Media. 2013;1:526.
24. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. Biomaterials Science: Anintroduction to materials in medicine. USA: Elsevier Academic Press. 2004:403-404