Silver nanoparticle-coated suture effectively reduces inflammation and improves mechanical strength at intestinal anastomosis in mice

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A B S T R A C T

Background: Our previous studies have revealed that silver nanoparticles (AgNPs) had anti-inflammatory properties. In this study, we coated AgNPs onto the surface of absorbable suture, to further explore their anti-inflammatory efficacy and potential clinical application using an intestinal anastomosis model.

Methods: Layer-by-layer deposition was used to coat AgNPs on absorbable sutures. Scanning electron microscopy (SEM) was conducted to observe the morphology and distribution of AgNPs on suture surface. 1 cm of either non-coated suture, suture coated with antibiotics or AgNPs-coated suture was placed on E. coli overlay of LB agar plates to test for bacterial inhibition. The respective sutures were then used for ileal anastomosis in mice. The anastomotic sites were harvested to investigate the degree of tissue inflammation and cell proliferation, as well as collagen deposition. Furthermore, burst pressure measurement was employed to test for mechanical properties.

Results: SEM observation indicated AgNPs could be immobilized and distributed on suture surface evenly. AgNPs-coated suture had the best in vitro anti-bacterial efficacy when compared with other groups. Subsequent immunohistochemistry in the intestinal anastomosis model showed significantly less inflammatory cell infiltration (macrophage and neutrophil) and better collagen deposition in the anastomotic tissue in the AgNPs-coated suture group. Burst pressure measurement in healed anastomosis further confirmed that AgNPs-coated suture had better mechanical properties.

Conclusion: Our study suggests that AgNPs-coated sutures can improve anastomosis healing due to better mechanical properties from reduced inflammation.

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The emergence of nanotechnology has made it possible to engineer some conventional materials into nano scale level, which exhibit new physiochemical and biomedical properties [1]. Among all the nanomaterials, silver nanoparticles (AgNPs) have been under the most intensive research in recent years. AgNPs have long been shown to have broad-spectrum antibacterial effects in many studies [2–5]. Furthermore, our previous studies also revealed that AgNPs had anti-inflammatory effects, both in a burn wound model, as well as in a periorteal adhesion model [6,7]. In addition to above models, the anti-inflammatory effect mediated by AgNPs can also be noted in other models, including contaminated wounds [8], ulcerative colitis [9], and chronic venous leg ulcers [10], as well contact dermatitis model [11]. Although the exact mechanisms of AgNPs-mediated anti-inflammatory effect still remain unknown, some studies have proposed that AgNPs can effectively reduce the infiltration of inflammatory cells, inhibit the production of inflammatory cytokines, and up-regulate the expression of matrix metalloproteinase (MMPs) [6–8].

Everyday, thousands of patients undergo surgical operations and sutures are used to repair tissues. The development of post-operative wound infection is a complication, which not only retards normal wound healing, but may also may induce life-threatening situations. In order to avoid the suture being a risk factor for wound infection, antibiotic-coated sutures have been introduced in the market in recent times. These sutures have been shown to have good antibacterial efficacy both in in-vitro, animal, as well as clinical studies [12–15]. Nonetheless, the prolonged use of antibiotics will eventually lead to bacterial resistance and increase organism virulence. Taking these and our previous research together [6,7], the addition of AgNPs on sutures may prove to be an excellent alternative. We therefore investigated this by immobilizing AgNPs on sutures and compared the anti-bacterial, anti-inflammatory and pro-healing properties against existing antibiotic-coated sutures in a mouse intestinal anastomosis model.
1. Materials and methods

1.1. Chemicals and silver nanoparticles solution preparation

Poly-diallyldimethylammonium chloride (PDADMAC) (MW = 200–350), poly-methacrylic acid (PMA) (MW = 9500) and silver nitrate (AgNO₃), as well as sodium chloride were purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA). The pH of above was set to 7 with 1 mM sodium acetate. Double distilled water was used to dilute the AgNO₃ solution.

Silver nanoparticle solutions were prepared by photo-induced reduction under UV lamp of silver nitrate in dilute solution of PMA. Briefly, AgNO₃ and PMA solutions were mixed volume for volume and then kept under UV for 4 hours to make solution A. The reduction of silver ions into AgNPs led to the appearance of pink color, which finally turned red. 1 mM PDADMAC solution was set as solution B. Both solution A and B were used to prepare AgNPs immobilisation onto the suture.

1.2. Sutures and immobilization of AgNPs to sutures

Absorbable suture coated with antibiotics (Vicryl Plus) and control suture (Vicryl) were purchased from Ethicon Ltd (USA). In each group, the same suture size (6–0) was used for anastomosis. Layer-by-layer deposition of AgNPs was employed to prepare AgNPs-coated suture from plain vicryl suture [16]. In brief, control suture was immersed successively into solution A and solution B and then rinsed in double distilled water and then left air-dried. The process was then repeated 20 times. The coated sutures were allowed to dry overnight at the end of deposition process before measurements with the spectrophotometer. AgNPs-coated sutures would also be visualised using scanning electronic microscope.

1.3. Scanning Electron Microscopy (SEM)

SEM imaging of AgNPs on surface of suture was examined for visualisation. The AgNPs-coated suture was cut into 1–2 mm. Suture was placed on a carbon film 300 mesh copper grid with the help of a syringe and dried prior to the microscopy. Imaging was photographed and recorded with Hitachi S4800 FEG-type scanning electron microscope (SEM), operating at 20 kV in vacuum to observe the morphology and distribution of silver on suture. For the elemental analysis, the electron microscope was equipped with an energy dispersive X-ray (EDAX) detector.

1.4. Antibacterial study to AgNPs solution and AgNPs-coated suture

Escherichia coli DH5α™ (E. coli, Invitrogen, USA) were used as test strains. Zone of inhibition assay was utilized to compare the antibacterial efficacy for both AgNPs solution and various sutures. Bacterial suspension of 10⁸ colony-forming units (CFU) was inoculated into 10 ml of LB soft agar to obtain a uniform bacterial overlay on LB agar plates. For the antibacterial test, 1 cm lengths of suture were placed on bacterial overlay of plates and gently pressed in. The plate was kept for incubation for 24 h at 37 °C. Inhibition zones were then observed and photographed every 24 hours for comparison [17,18].

1.5. Animal experiment

6–7 weeks old C57BL/6 N mice, weighing 21 ± 3 grams, were obtained from the Laboratory Animal Unit, The University of Hong Kong. The experimental protocol was approved by the Committee of the Use of Live Animals in Teaching and Research, The University of Hong Kong (CULATR 1599–08). Anesthesia for experimentation was performed by intra-peritoneal injection of pentobarbital sodium solution (Abbott Laboratories, IL, U.S.A) at a dose of 50 mg/Kg. Mice were randomized into three groups, AgNPs-coated suture, antibiotics suture and control suture groups (n = 6). All animal studies were performed by the same person under the same experimental environment.

After laparotomy, the ileum 2 cm from the cecum was cut open with scissors. Any spillage of intestinal contents was removed by suction. Intestinal defect was closed using single layered, interrupted anastomosis using 6–0 suture in each group. The abdominal wall was

Table 1

<table>
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<tr>
<th>Antibody against</th>
<th>Abbreviation</th>
<th>Dilution</th>
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<tbody>
<tr>
<td>Macrophage</td>
<td>F4/80</td>
<td>1:100</td>
<td>Rat</td>
<td>Santa Cruz</td>
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<tr>
<td>Neutrophil</td>
<td>PMN</td>
<td>1:200</td>
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then closed with running suture in both peritoneal-muscular layer and skin layer. At the end of surgery mice were resuscitated and allowed free access to water and liquid diet when awake.

1.6. Immunohistochemistry for inflammatory cells

Anastomosed intestinal segments in each group were harvested on post-operative day 3, formalin-fixed and embedded in paraffin and sectioned (4 um thick). The sections were deparaffinized and rehydrated, then the endogenous peroxidase was quenched by treatment in 3% hydrogen peroxide/methanol. Afterwards, they were incubated for 1 hour at room temperature with blocking solution containing 5% normal goat serum (Dako Bioresearch, USA). For antigen retrieval of cells and proteins staining, the sections were blocked for nonspecific binding solutions containing 5% concentration of normal goat serum before primary antibodies to stain for neutrophils or macrophages were added (Table 1). The sections were incubated overnight at 4°C in a dark room before rinsing in phosphate-buffered saline (PBS), and incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, CA, USA) for 2 hours. Positive signals were developed using DAB (3, 3′-diaminobenzide tetrahydrochloride) and counterstained with haematoxylin. The degree of inflammation, as defined by the number of inflammatory cells, was assessed subjectively by one author who was blinded to the groups.

![Fig. 2. SEM shows the ultra-structure of various sutures surface. (A) The distribution and density of AgNPs on suture after one cycle or four cycles of coating, as well as their content on suture; (B) The morphology and distribution of AgNPs on surface of suture after 20 cycles of coating in solutions, in comparison to antibiotic-coated sutures and control.](image-url)
1.7. Masson staining

Anastomosed intestinal tissues were collected and processed as previously mentioned. Collagen fibers were stained using Masson Trichrome method to observe the density and distribution of newly deposited collagen around the anastomosis. Briefly, the rehydrated sections were mordant in preheated Bouin’s solution at 56 °C for 12 minutes, followed by washing in tap water to remove yellow dye. Slides were inserted in working Weigert’s iron hematoxylin solution for 5 minutes and followed by rinsing. They were in tandem stained with Biebrich scarlet-acid Fuchsin solution, phosphotungstic/phosphomolybdic acid solution and Aniline Blue solution for 5 minutes respectively. Finally the slides were put in acetic acid (1%) for 2 minutes, followed by rinsing, dehydration with alcohol (from 70%, 95% to 100%), clearance in xylene and mounted. Images were observed and photo taken under microscopy.

1.8. Burst pressure measurement at anastomotic site

Burst pressure measurement was same as previous described [19–21]. Briefly, measurements were evaluated by two investigators blinded to the treatment group. Burst pressure was measured in a 5 cm segment of ileum containing the anastomosed segment harvested on postoperative day 7. Both ends of the resected ileum tissue were ligated with 3–0 sutures. A venous catheter was inserted at one end and connected to a 50 ml syringe containing double distilled water. The

Fig. 3. The effect of AgNP-coated suture on Escherichia coli DH5α™. (A) Comparisons of the inhibition zones effected by normal suture, antibiotic-coated suture and AgNP-coated suture, photographed on day 1, 5 and 9. (B) Graph plot showing the ratio of inhibition zone at various time points compared to day 1 in antibiotic suture group and AgNPs-coated suture group.
other end of resected ileum tissue was connected and fixed to a pressure transducer [Fig. 1]. The water was evenly injected by hand and pressure measurement was performed until bursting was seen. The maximal values were accepted as anastomotic bursting pressure.

1.9. Statistics

Statistical analyses were conducted by using Student’s paired t-test. A p value of < 0.05 was considered statistically significant.

2. Results

2.1. AgNPs could be immobilized and distributed evenly on surface of suture

EDAX spectrum and SEM imaging confirmed the presence of carbon (C), oxygen (O), calcium (Ca), chloride (Cl) and silver (Ag). Regarding to metallic component, the amount of Ag was much more than that of Ca. We next investigated and compared the morphology and distribution of Ag in AgNPs-coated sutures with different cycles of coating (1 cycle vs. 4 cycles). The elemental maps in EDAX spectrum indicated that the number of cycles of Ag immobilisation correlated positively with the amount of Ag present [Fig. 2A].

Despite these, we observed that AgNPs could only be immobilized onto superficial surface of sutures, their morphology was dense and distributed evenly, instead of forming clusters [Fig. 2B].

2.2. AgNPs-coated suture had better in-vitro anti-bacterial efficacy

Our previous study already revealed that AgNPs in solution form had excellent antibacterial efficacy [6]. We therefore asked if this effect could still be seen once AgNPs were immobilised onto the surface of suture. In order to assess the anti-bacterial efficacy of the suture in each group, inhibition assay for E. coli was performed. Here, we found that no inhibition zone could be seen in the control group for each time point. In both antibiotics and AgNPs-coated suture

Fig. 4. Histological evaluations of inflammatory response during healing of intestinal anastomosis. (A) Neutrophil infiltration on post-anastomosis day 3 in each group; (B) Macrophage infiltration on post-anastomotic day 7 in each group.
groups, significant inhibition zone were noted [Fig. 3A]. However, the areas of inhibition in both groups would decrease with time (days 1, 5 and 9). Nonetheless, when compared with antibiotics suture group, the inhibition zone in AgNPs-coated suture groups still remained significant on day 9.

In order to further quantify the antibacterial efficacy, we chose 6 time points (day 1, 2, 3, 5, 7, 9) and photographed the inhibition zones in antibiotics and AgNPs-coated suture groups. The areas of the inhibition zones at various time points were then compared to those on day 1 and expressed as ratios. The results here showed that the antibiotic-coated suture started to lose its efficacy on day 5, while the AgNPs-coated suture continued to inhibit bacterial growth up to day 9 [Fig. 3B].

2.3. AgNPs-coated suture demonstrated in vivo anti-inflammatory efficacy

We next investigated anti-inflammation efficacy of AgNPs-coated suture in an intestinal anastomosis model. Segments of intestinal anastomosis in each group were harvested on post-surgical day 3 to stain for neutrophil infiltration. Here, we observed significantly more neutrophil infiltration around anastomotic tissues in the control suture group, as compared to antibiotics group and AgNPs-coated group. The AgNPs-coated group had the least neutrophil infiltration [Fig. 4A]. As neutrophil infiltration is an early phase biological event during the inflammatory response, we next investigated and compared macrophage infiltration in anastomotic tissues samples on post-surgical day 7. The results again showed similar trend to neutrophil infiltration, with more macrophage infiltration in the control group and least in the AgNPs-coated group [Fig. 4B]. Taken together, this would suggest that AgNPs-coated suture could effectively decrease the inflammatory cell infiltration.

2.4. Healed tissue treated with AgNPs-coated suture had better collagen deposition and better mechanical properties

As the AgNPs-coated suture induced better anti-inflammatory efficacy, we next wanted to determine if more extracellular matrix (ECM) would still be produced. Masson staining was conducted to explore collagen deposition, the main ECM in intestinal wall. In samples taken on post-surgical day 7 and 14, we found collagen deposition in both the antibiotics and AgNPs-coated groups but very little in amount in the control group. In contrast to the antibiotics suture group, more collagen deposition again could be seen in AgNPs-coated suture group. On day 14, AgNPs-coated group had significantly more collagen than other two groups [Fig. 5].

More collagen deposition during tissue healing after anastomosis should result in the better mechanical strength. In order to confirm

![Fig. 5. Masson Trichrome staining photographs of post-anastomotic intestinal wall tissue on post-anastomosis day 7 and day 14. Staining showed the distribution and density of collagen protein in skin dermal layer. Under this staining, collagen protein was stained to blue, nuclei were staining to black and background (muscle, cytoplasm and keratin).](image-url)
problem of drug resistant strains. We therefore hypothesized that we could produce an alternative suture product which would have at least an equivalent, if not better efficacy.

Based on our previous research, we expected both antibacterial and anti-inflammatory actions from silver nanoparticles and indeed this was proved to be true. We showed in our in-vivo experiment that AgNPs-coated sutures reduced significantly the local inflammatory response. The reduction of inflammation however did not result in poor healing. As seen clearly in Fig. 5, there was in fact increased collagen production in the AgNP-coated suture group in the early phase of healing, as well as better mechanical strength in later phase of healing. Taken together, it would suggest that uncontrolled inflammation may indeed impair normal healing, perhaps due to excessive scar tissue formation.

The main difficulty in this study was how to incorporate AgNPs into suture material. This was overcome by immobilising using a layer by layer method. As shown in Fig. 2, the amount of AgNPs coated on the suture increased with an increase in immobilisation cycles. What we did not evaluate in this study was the optimum amount of AgNPs needed to have the best efficacy. This will be performed in future research. Furthermore, from the EM photographs, we could observe that after coating, although AgNPs were seen to be evenly distributed on the surface of suture, the deep grooves between suture strands were not coated. The filling of these deep grooves should be attained so as to maximize the full potential of silver nanoparticles. Thus, a better and more refined coating method will need to be employed. Another study which needs to be carried out in the future would be to see whether monofilament sutures can also be coated. The use of subjective assessment for the degree of inflammation was another shortcoming, which could be rectified by counting of inflammatory cells from random visual fields.

The use of any nanomaterial always raises the question of potential toxicity. Thus far, there is no concrete evidence in the literature, as well as by our research, of any significant cytotoxicity induced by AgNPs, especially in this small amount. Furthermore, when used in vivo, body fluids in the surrounding environment could dilute AgNPs and this natural buffer system could help decrease potential toxicity. In conclusion, we have shown that AgNP-coated suture could provide an ideal environment to promote intestinal anastomotic healing. Further research is needed to realise its clinical potential.

References


