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Long-term anti-inflammatory efficacy in intestinal anastomosis in mice using silver nanoparticle-coated suture

Xuelai Liu [a,1,2], Peng Gao [b,1], Juan Du [c], Xin Zhao [d], Kenneth K.Y. Wong [a,*]

[a] Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[b] Department of Surgery, Harbin Children's Hospital, Harbin, China
[c] Department of Medicine, Changchun Central Hospital, Changchun, Jilin Province, China
[d] Interdisciplinary Division of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong SAR, China

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ABSTRACT

Background: In our previous study, we coated silver nanoparticles (AgNPs) onto the surface of absorbable braided suture using layer-by-layer deposition and demonstrated significant anti-inflammatory property during the early phase of intestinal anastomosis healing in mice. The present study aimed to further investigate the long-term anti-inflammatory efficacy.

Methods: AgNP-coated suture, antibiotic coated suture, and normal suture were respectively used for single layered, interrupted intestinal anastomosis. The anastomotic segments in each group were harvested on day 14, day 21, and day 28 postoperation and investigated for the degree of inflammation by cell infiltration and expression of cytokines as well as collagen deposition.

Results: When compared with *the* control groups, the AgNP-coated suture group showed better histological appearance in the intestinal anastomotic segments at each time point. Immunohistochemistry staining and quantitative evaluation further indicated less macrophage infiltration and decreased production of IL-6, IL-10, and TNF-α ($p < 0.05$). Masson staining showed normal collagen deposition and remodeling at intestinal anastomotic tissue in the AgNP-coated suture group.

Conclusion: Our study shows that AgNP-coated suture provides better long-term anti-inflammatory efficacy and ideal tissue remodeling in intestinal anastomosis. Despite these findings, clinical trials are still needed for evaluation before medical application.

Silver nanoparticles (AgNPs) are nanoscale size pure silver containing 20–15,000 silver atoms and measuring less than 100 nm in diameter [1,2]. Many studies have suggested they have excellent in vitro anti-inflammatory properties. Furthermore, the anti-inflammatory action induced by AgNPs was found to accelerate tissue repair and regeneration in various animal models, including ulcerative colitis and skin contact dermatitis [3,4]. Our previous studies also supported these findings in skin wound healing [5], and in the reduction of peritoneal adhesions [6], because of the inhibition of inflammatory cell infiltration and suppressed production of proinflammatory cytokines.

Based on these, we further tested the coating of Vicryl sutures (Polyglactin 910) (Ethicon, Somerville, NJ) with AgNPs using layer-by-layer method. We found that AgNP-coated suture had more prolonged in vitro antibacterial effect when compared to commercial antibiotic-coated suture. AgNP-coated suture was shown also to have excellent anti-inflammatory action with better mechanical strength at anastomotic site in an intestinal anastomosis model in mice [7]. Despite these exciting findings, the efficacy during the late phase of intestinal tissue healing was not known. The aim of the present study was thus to further investigate the long-term anti-inflammatory efficacy induced by AgNP-coated suture.

1. Materials and methods

1.1. Preparation of silver nanoparticles

Polymethacrylic acid (PMA), polydiallyldimethylammonium chloride (PDADMAC), silver nitrate ($AgNO_3$), and sodium chloride were purchased from Sigma-Aldrich Ltd. (St. Louis, MO). All solutions were adjusted to a value of pH 7 with 1 mM sodium acetate and stored at room temperature.

The preparation of silver nanoparticle (AgNPs) solutions was same as previously described [7]. Briefly, equivalent volume of $AgNO_3$ and

* Corresponding author at: Department of Surgery, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong SAR, China. Tel.: +852 22553486; fax: +852 28173155.
E-mail address: kkywong@hku.hk (K.K.Y. Wong).
[1] Both authors contributed equally to this work.
[2] Current address: Department of Surgery, The Second Hospital, HeBei Medical University, ShiJiaZhuang City, Hebei Province, China.

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PMA solutions were mixed followed by photoinduced reduction under UV lamp for 4 h to prepare solution A. The color of solution A would change from pink to red because of the formation of AgNPs. PDADMAC solution was diluted into 1 mM working solution with 1 mM sodium acetate and set as solution B. Solutions A and B were used for fabrication of AgNP-coated sutures.

1.2. Fabrication of AgNP-coated sutures

6-0 Vicryl® suture (Ethicon) and Vicryl Plus® (with antibiotics) were purchased from Ethicon Ltd. and set as controls. Layer-by-layer deposition method was used to fabricate AgNP-coated Vicryl sutures as previously described [7,8]. The AgNP-coated sutures were dried overnight after 20 circles of coating. The amount of AgNPs coated was measured with spectrophotometer and the distribution of AgNPs immobilized on suture was studied by scanning electron microscope. The three groups of sutures were stored at room temperature before animal experiments.

1.3. Animal experiment

The experimental protocol in this study was approved by the Committee of the Use of Live Animals in Teaching and Research, The University of Hong Kong (CULATR 1599-08). C57BL/6 N mice, weighing between 20 ± 2 g and ranging in age from 6 to 8 weeks, were provided by the Laboratory Animal Unit, The University of Hong Kong. Mice were randomized into three groups (AgNP-coated suture, antibiotic-coated suture and normal suture groups, 7 mice/group). Intraperitoneal injection of pentobarbital sodium solution (Abbott Laboratories, Abbott Park, IL) at a dose of 50 mg/kg was used for anesthesia. All animal experiments were performed by the same investigator. For the intestinal anastomosis, the ileum (2 cm from the cecum) was cut with scissors after the abdomen was opened. The ends were then closed with single layer, interrupted anastomosis using 6-0 suture in each group. The abdomen was then closed by mass closure. All the mice were given analgesia and allowed free access to water and diet after surgery.

1.4. Histological staining

The animals were sacrificed on day 14, day 21 and day 28 postoperation and the anastomotic site in each group was harvested. The specimens were formalin-fixed and embedded in paraffin. 4 μm-thick sections were taken, dewaxed and rehydrated for hematoxylin and eosin (H&E) staining.

For immunohistochemistry staining, endogenous peroxidase was quenched by 3% hydrogen peroxide/methanol after sections were deparaffinized and rehydrated, followed by incubation at room temperature with blocking solution containing 5% normal goat serum (Dako Bioresearch, USA). For antigen retrieval in tissues, the sections were blocked for nonspecific binding solutions containing 5% concentration of normal goat serum before primary antibody was added [7]. The sections were incubated overnight before rinsing in phosphate-buffered saline (PBS), and incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Positive signals were developed using DAB (3,3′-diaminobenzidine) and counter-stained in hematoxylin solution, followed by photographing microscope. The degree of inflammatory cell infiltration was assessed by a researcher who was blinded to the experimental groups.

1.5. Masson staining

The anastomotic segments from each group were collected and processed as above. Masson trichrome staining was performed to observe density and distribution of collagen at anastomotic tissue. In brief, the rehydrated sections were mordant in preheated Bouin's solution at 56 °C for 10–15 min, followed by washing in tap water to remove extra dye. Slides were immersed in working Weigert's iron hematoxylin solution for 5 min followed by rinsing. They were in tandem stained with Biebrich scarlet-acid Fucshin solution, phosphotungstic/phosphomolybdic acid solution and Aniline Blue solution for 5 min respectively. Finally slides were put in acetic acid (1%) for 2 min, followed by rinsing in tap water, dehydration with alcohol (from 70%, 95% to 100%), clearance in xylene and mounted.

1.6. Morphometric evaluation

Digital software (Image-Pro plus 6.0; Media Cybernetics) was used to quantify immunohistochemistry staining in anastomotic intestinal tissues. The average optical density in stained area was determined in five random images with 400× magnification. The numeric data obtained from the image analysis were exported for statistical analysis. The ratio between average optical density of tissue in each group and normal ileum was calculated for evaluation.

1.7. Statistics

Statistical analyses were conducted using Student's paired t test. A p value <0.05 was considered significant. The results showed the average value ± standard deviation.

2. Results

2.1. Anastomosed intestinal tissue using AgNP-coated suture showed better macroscopic appearance at the late phase of healing

As edema and hyperemia are the most frequent pathological reactions after tissue inflammation, we assessed and recorded the gross morphology of the intestinal suture site at each time point when the abdomen was reopened. Although there was no significant peritoneal adhesion in each group, we did find less edema and hyperemic reaction in the AgNP-coated suture group in each time point when compared with the antibiotic suture and control groups, especially at postoperative day 28 time-point. Better macroscopic morphology with appearance resembling normal intestinal tissue could be observed in the AgNP-coated suture group in contrast to other two groups (Fig. 1). These would suggest, at least at the macroscopic level, that the AgNP-coated suture group had resulted in reduced inflammatory responses at the late phase of anastomosis and could contribute to “more physiological” intestinal tissue healing after wounding.

2.2. Reduced inflammatory response with less macrophage infiltration seen in the AgNP-coated suture group

Segments of intestinal anastomosis in each group were harvested and serial sections were subjected for histological evaluation. H&E staining showed that proliferated serosal tissue was found in the control, antibiotics suture and AgNP-coated suture groups. Nonetheless, the proliferative tissue response was less in the AgNP-coated suture group in each time point, in contrast to the control or antibiotics suture groups (Fig. 2A) (images from postsurgical day 14 and day 28 were not shown).

We next investigated the anti-inflammatory efficacy of AgNP-coated suture. Immunohistochemistry staining was conducted on the three time points (postsurgical day 14, day 21 and day 28) for inflammatory cell infiltration. We did not find neutrophil infiltration around anastomotic tissues in any of the time points for each group. However, the AgNP-coated suture group had the least macrophage infiltration when compared to the control and antibiotics-suture group, and showed the most resemblance to normal ileum tissue (Fig. 2A). These results suggested that AgNP-coated suture could effectively decrease inflammatory cell infiltration over the long term. In order to specifically quantify the differences in each group, we calculated the average optical density ratio of tissue in each group to normal ileum. Our results confirmed