Arsenic trioxide-eluting electrospun nanofiber-covered self-expandable metallic stent reduces granulation tissue hyperplasia in rabbit trachea

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Abstract

Stent-related granulation tissue hyperplasia is a major complication that limits the application of stents in airways. In this study, an arsenic trioxide-eluting electrospun nanofiber-covered self-expandable metallic stent (ATO-NFCS) was developed. Poly-L-lactide-caprolactone (PLCL) was selected as the drug-carrying polymer. Stents with two different ATO contents (0.4% ATO/PLCL and 1.2% ATO/PLCL) were fabricated. The in vitro release in simulated airway fluid suggested that the total ATO release time was 1 d. The growth of human embryonic pulmonary fibroblasts (CCC-HPF-1), normal human bronchial epithelial cells and airway smooth muscle cells was inhibited by ATO. When embedded in paravertebral muscle, the nanofiber membrane showed good short-term and long-term biological effects. In an animal study, placement of the ATO-NFCS in the trachea through a delivery system under fluoroscopy was feasible. The changes in liver and kidney function 1 and 7 d after ATO-NFCS placement were within the normal range. On pathological examination, the heart, liver, spleen, lungs and kidneys were normal. The effectiveness of the ATO-NFCS in reducing granulation tissue hyperplasia and collagen deposition was demonstrated in the rabbit airway (n = 18) at 4 weeks. The present study preliminarily investigated the efficacy of the ATO-NFCS in reducing granulation tissue formation in the trachea of rabbits. The results suggest that the ATO-NFCS is safe in vivo, easy to place, and effective for the suppression of granulation tissue formation.

1. Introduction

Metal tracheal stents, as a fast and effective method for treating tracheal stenosis, can quickly support the narrow lumen and restore airway patency [1, 2]. However, stent-related complications, such as stent embedment, granulation tissue hyperplasia, infection and stent migration, should not be ignored [3]. These complications limit the application of metal tracheal stents in benign tracheal stenosis. Coatings on metal stents can prevent proliferating granulation tissue from growing through the mesh, but there the proliferation of granulation tissue still occurs at both ends of the stent [4]. Therefore, inhibiting the proliferation of granulation tissue at both ends of the stent is the key to solving this problem. Vascular drug-eluting stents, fabricated by coating the drug on the bare stent wire for continuous
release of the drug to inhibit proliferation, are used to inhibit the proliferation of the vascular intima and have achieved good results [5, 6]. Also, a series of biodegradable stent were designed to reduce the hyperplasia of intima [7, 8]. However, animal experiments with tracheal drug-eluting stents are ongoing, and the proliferation of granulation tissue through the stent mesh is not uncommon [9]. Therefore, there is an urgent need for a drug-eluting covered tracheal stent that can effectively inhibit granulation tissue proliferation.

Arsenic trioxide (ATO) has been used as a medicine for 2400 years and has significant anti-tumor and anti-inflammatory effects [10]. It is widely used in the treatment of leukemia and many advanced tumors [11]. The mechanism of action of ATO includes directly inducing apoptosis, inhibiting cell differentiation, inhibiting cell proliferation, and inhibiting angiogenesis [12]. Compared with sirolimus (rapamycin)-eluting and paclitaxel-eluting stents in cardiovascular, ATO-eluting stent has the strong ability to induce smooth muscle cell apoptosis while sirolimus (rapamycin)-eluting and paclitaxel-eluting stents are maintain a balance between apoptosis and proliferation [5, 13]. Furthermore, the application of paclitaxel-eluting stents in rabbit trachea were surprisingly negative and causing significant lesions with destruction in all layers of the trachea. The main components of tracheal granulation tissue caused by stents are fibroblasts and neovascularization. The inhibitory effect of ATO on fibroblasts has been demonstrated in cell culture [14–16]. Therefore, ATO has the potential to inhibit granulation tissue hyperplasia in the trachea.

Electrospinning is a simple technique for making nanofibers, which can be done with a variety of materials. Nanofiber membranes made by electrospinning have a multivoid structure and a large specific surface area and are good carriers for drug release [17]. Poly-L-lactide-caprolactone (PLCL) is a biodegradable polymer with good biocompatibility [18] that can be electrospun into nanofiber membranes. This material has degradability, drug permeability and nontoxic degradation products and has been used in the manufacture of surgical sutures and sustained-release drug carriers. There have been many studies on the application of in-body materials and tissue engineering scaffold materials [19–21]. In this study, we used PLCL to fabricate nanofiber membranes by electrospinning technology for the design of an ATO-eluting nanofiber-covered self-expandable metal stent (ATO-NFCS).

Based on the above information, we designed an ATO-NFCS loaded with different concentrations of ATO and examined the safety and efficacy of the stents through physicochemical characterization, in vitro drug release studies, biocompatibility analysis and animal experiments.

2. Materials and methods

2.1. Ethics statement

This study was carried out with the Guidelines on the Care and Use of Laboratory Animals issued by the Chinese Council on Animal Research and the Guidelines of Animal Care. The protocol was approved by the Committee on the Ethics of Animal Experiments of Zhengzhou University. Animal studies were conducted at Henan Key Laboratory for Pharmacology of Liver Disease (No. SYDW 2019 0407).

2.2. Materials

ATO for injection was purchased from Shuanglu Pharm Co., Ltd (Beijing, China) Poly(L-lactide-co-caprolactone) (PLCL, lactic acid: caprolactone ratio = 1:1, intrinsic viscosity = 3.9 dl g⁻¹) from Daigang Biomaterial Co., Ltd. (Jinan, China). Dichloromethane (DCM) and N,N-dimethylformamide (DMF) were supplied by Tianjin Kemiou Chemical Co., Ltd. (Tianjin, China). Uncovered self-expandable metallic stent (SEMS) (20 mm × 8 mm) was purchased from Nanjing micro-tech Co., Ltd. (Nanjing, China). Mucin was purchased from Kuer chemistry Co., Ltd. (Beijing, China). Human embryonic pulmonary fibroblasts (CCC-HPF-1) and normal human bronchial epithelial cells (NHBE) were purchased from Beijing Oligobio. Airway smooth muscle cells (SMC) were purchased from Wuhan Procell. Dulbecco’s modified Eagle’s medium (DMEM) (high glucose), fetal bovine serum (FBS) and phosphate-buffered saline (PBS), were purchased from Biological Industries (Israel). Cell counting kit-8 (CCK-8) assay kit was purchased from Dojindo (Japan).

2.3. Preparation of nanofiber solutions

To obtain the solution for electrospinning, PLCL was dissolved in a mixture of DCM and DMF with a ratio by volume of 7:3 at a concentration of 5% (w/v), and the ATO was dissolved in deionized water. Then, we obtained solutions of 0.4% ATO/PLCL (w/w) and 1.2% ATO/PLCL (w/w) by mixing those two solutions. The mixed solution was stirred with a magnetic mixer for an hour before electrospinning.

2.4. Fabrication of the ATO-NFCS

The electrospinning system used in this study is shown in figure 1(a). The parameter for electrospinning as describe in our previous study [22]. An SEMS was aligned on the roller collector to collect the nanofiber. A direct current voltage of 20 kV was applied between the syringe tip and the collector. The distance between the tip and the grounded collector was 18 cm. To control the thickness of the fiber mesh, 1.8 ml of solution was supplied for every NFCS. Once the electrospinning process was finished, the NFCS was well
dried and stored in an airtight container at $-4 \, ^\circ C$ for further use.

2.5. Scanning electron microscopy (SEM)
The NFCS was first coated with platinum, and then the surface morphology was imaged by SEM at an accelerating voltage of 10 kV. ImageJ was used to measure the diameter of the nanofibers.

2.6. Water contact angle
To verify the wettability of the NFCS, the nanofiber membrane was stripped to measure the water contact angle. The contact angle was measured using the sessile drop method by dispensing 2 $\mu l$ of deionized water onto the surface of the membrane at room temperature.

2.7. Drug release of the ATO-NFCS in vitro
The in vitro release of ATO from the ATO-NFCS was determined using an inductively coupled plasma mass spectrometer. For each measurement, the ATO-NFCS was soaked in 20 ml of simulated airway fluid (SAF) for a predetermined time. The components of SAF [23] were as follows: magnesium chloride (0.095 g/L), sodium chloride (6.019 g l$^{-1}$), potassium chloride (0.298 g l$^{-1}$), disodium hydrogen phosphate (0.126 g l$^{-1}$), sodium sulfate (0.063 g l$^{-1}$), calcium chloride dehydrate (0.368 g l$^{-1}$), sodium acetate (0.574 g l$^{-1}$), sodium hydrogen carbonate (2.604 g l$^{-1}$), sodium citrate dehydrate (0.097 g l$^{-1}$), and porcine stomach mucin (0.6 g l$^{-1}$). The samples were kept on a rotating shaker set to 100 rpm at 37 $^\circ C$. At specified periods, 1 ml of the solution was collected and replaced with the same volume of SAF. All the solution samples were stored at 4 $^\circ C$ until analysis.

2.8. Growth of cells on the ATO/PLCL nanofiber membrane
To evaluate the cytotoxicity of the ATO-NFCS, CCC-HPF-1, NHBE and airway SMC were cultured on ATO/PLCL nanofiber membranes with different concentrations of ATO. The Cell Counting Kit-8 assay was chosen to detect the effect on cell proliferation. Cells were cultured in DMEM supplemented with 10% (v/v) FBS and were maintained in a humidified atmosphere of 5% CO$_2$ and 95% air. Approximately 10$^4$ cells per well were seeded on the ATO/PLCL nanofiber membrane samples in a 24-well plate and incubated for 1, 3 and 5 d. The medium was replaced every day. All the membrane samples used for cell culture were sterilized by ultraviolet light on both sides. All tests were performed with at least three replicates.

2.9. Biological response to the ATO/PLCL nanofiber membrane
To evaluate the biological response to the ATO/PLCL nanofiber membrane, membrane samples 10 $\times$ 10 mm$^2$ in size from each group were embedded bilaterally in the paravertebral muscles. The time points of 2 weeks and 12 weeks were chosen to obtain samples representing the short-term and long-term responses, respectively. The biological response was evaluated through pathological findings. The degradation of the nanofiber membrane was also observed.

2.10. Application of the ATO-NFCS in rabbits
A 5-F delivery system was chosen to insert the stent. The process of loading the delivery system with the stent was as follows. First, the outer sheath of the delivery system was withdrawn. Second, the ATO-NFCS was compressed onto the core of the delivery system. Finally, the outer sheath was pushed back to contain the stent, completing the process of stent loading. Upon withdrawal of the outer sheath, the stent expanded due to its self-expandable nature.

A total of 18 New Zealand rabbits (offered by Hualan Biological Engineering Co Ltd.) were randomly divided into three groups, with six rabbits in each group. A stent was placed in all rabbits under the guidance of fluoroscopy. Rabbits in group A received a 1.2% ATO-NFCS, rabbits in group B received a 0.4% ATO-NFCS, and rabbits in group C received an NFCS without ATO.

After anesthetization, a catheter with a guide wire was inserted into the airway of the rabbit under the guidance of fluoroscopy. Then, the guide wire was withdrawn, and 0.5 ml of contrast agent was injected with 0.5 ml of 0.9% saline to show the tracheobronchial tree. Next, the catheter was exchanged for the stent delivery system using the guide wire. Once the distal stent arrived at the level of the trachea almost 2 cm above the carina, the stent was released slowly. Finally, the delivery system was withdrawn, and the procedure was finished.

All rabbits were maintained in single cages with free access to standard food and water in a fully controlled environment at a room temperature of $22 \pm 2 \, ^\circ C$ and relative humidity of 45 $\pm$ 15% under a 12 h light/dark cycle.

2.11. Blood test
To assess the safety of the ATO-NFCS, blood markers of liver and kidney function were analyzed. Liver function markers included alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL). Kidney function markers included blood urea nitrogen (BUN), serum creatinine (Scr) and uric acid (UA). Two milliliters of blood were collected from each rabbit through the ear vein before stenting and 1 and 7 d after stenting (figure 1(b)). After centrifugation of the blood sample, serum was obtained and stored at $-80 \, ^\circ C$ for further analysis.

2.12. Pathological evaluation
The rabbits were sacrificed at 1 and 4 weeks after stent placement by the inhalation of pure carbon dioxide.
Figure 1. Schematic diagram of ATO-NFCS fabrication and the animal study. (a) PLCL was dissolved in a mixture of DCM and DMF with a ratio by volume of 7:3 at a concentration of 5% (w/v). ATO was dissolved in deionized water to obtain solutions of 0.4% ATO/PLCL (w/w) and 1.2% ATO/PLCL (w/w) for ATO-NFCS fabrication. (b) Blood was drawn before stenting and 1 and 7 d after stenting. The rabbits underwent computed tomography and were sacrificed at 1 and 4 weeks, as scheduled.

2.13. Statistical analysis
Differences between groups were analyzed by analysis of variance, as appropriate. Post hoc comparisons were performed using the Bonferroni method. Fisher’s exact test and the chi-squared test were used to compare categorical variables between groups. P < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software (version 21.0; SPSS, Chicago, USA).

3. Results
3.1. Physicochemical characterization of the ATO-NFCS
General images of all three NFCSs are shown in figure 2(a). The PLCL NFCS had a smooth surface. Compared with the PLCL NFCS, the surface of the ATO-NFCS was rougher and increased in roughness with increasing ATO content. As shown in figure 2(b), the surface morphology of the nanofiber membrane and the corresponding diameter of the nanofibers were observed using SEM. Particles were observed on the surface of the ATO/PLCL nanofiber membrane, indicating that ATO was successfully loaded. The surface of the 1.2% ATO/PLCL nanofiber membrane had more particles than that of the 0.4% ATO/PLCL nanofiber membrane. However, the surface of the PLCL nanofiber membrane did not exhibit particles. The observed diameter of the PLCL nanofibers was 539.2 ± 96.9 nm, and that of the 0.4% ATO/PLCL and 1.2% ATO/PLCL nanofibers was 563.5 ± 80.1 nm and 542.4 ± 90.9 nm, respectively (figure 2(c)).
Figure 2. Physicochemical characterization of the ATO-NFCS. (a) General images of the NFCS, 0.4% ATO-NFCS, and 1.2% ATO-NFCS. (b) Representative SEM images of the NFCS, 0.4% ATO-NFCS, and 1.2% ATO-NFCS. Mag: 2000x, 4000x. Bar: 10 µm. (c) Nanofiber diameter distribution determined from SEM images of the NFCS, 0.4% ATO-NFCS, and 1.2% ATO-NFCS (n = 3). (d) Water contact angle of the outer and inner surface of the NFCS, 0.4% ATO-NFCS, and 1.2% ATO-NFCS (n = 3). (e) ATO release from the 0.4% ATO-NFCS and 1.2% ATO-NFCS in SAF (n = 3).

As shown in figure 2(d), the water contact angle of the inner surface of the nanofiber membranes consisting of PLCL, 0.4% ATO/PLCL and 1.2% ATO/PLCL was 125.9 ± 2.2, 117.4 ± 7.0 and 118.2 ± 5.0, respectively. The water contact angle of the outer surface of the PLCL, 0.4% ATO/PLCL and 1.2% ATO/PLCL membranes was 114.6 ± 5.3, 115.9 ± 5.1 and 108.2 ± 2.9, respectively. The angle on both sides of the PLCL and ATO/PLCL nanofiber membranes was larger than 90°. These results indicate that both sides of all three kinds of nanofiber membranes are hydrophobic.

The results of ATO release in vitro are shown in figure 2(e). In the first 3 h, the ATO-NFCS showed a burst release. Over the next 3 h, the amount of ATO released decreased, and at 24 h, the cumulative amount of ATO released peaked; almost the same amount of ATO release was observed at 48 h and 72 h.

3.2. Cytocompatibility and histocompatibility of the ATO-NFCS

The growth of CCC-HPF-1, NHBE and SMC on the surface of the nanofiber membrane was examined (figures 3(a)–(c)). On day 1, the difference of optical
Figure 3. Cytocompatibility and histocompatibility of the ATO-NFCS. (a)-(c) Growth of CCC-HPF-1, NHBE and SMC on the surface of PLCL, 0.4% ATO/PLCL, and 1.2% ATO/PLCL nanofiber membranes (n = 3). P-values < 0.05 (*), < 0.01 (**), < 0.001 (***) and < 0.0001 (****). (d) Degradation of PLCL, 0.4% ATO/PLCL, and 1.2% ATO/PLCL nanofiber membranes in the trachea at 1 week and 4 weeks. (e) Biological response to and degradation of PLCL, 0.4% ATO/PLCL, and 1.2% ATO/PLCL nanofiber membranes at 2 weeks and 12 weeks after being embedded in paravertebral muscle (n = 3). HE staining, 200x.

The paravertebral muscle experiment showed that the nanofiber membrane was surrounded by mild fibrous tissue, inflammatory cells and neovascularization at 2 weeks (figure 3(d)). Layers of adipocytic infiltration were also observed in the surrounding tissue at 12 weeks. These results indicate that the nanofiber membrane elicits good short-term and long-term biological responses. After being embedded for 2 weeks, the degradation of the PLCL was observed in the form of strips of flaky cracks in all three kinds of nanofiber membranes. The degradation was more severe at 12 weeks. Compared with the PLCL nanofiber membrane, the tissue surrounding the ATO/PLCL membranes had fewer...
Figure 4. General evaluation of the ATO-NFCS in the rabbit trachea. (a) The ATO-NFCS was loaded and released through a 5-F delivery system. (b) Process of ATO-NFCS placement under the guidance of fluoroscopy in rabbits. (c) Anatomical observation of the trachea and outer surface of the NFCS, 0.4% ATO-NFCS, and 1.2% ATO-NFCS. (d) Cross-section of the trachea at the proximal end of the stent showing granulation tissue hyperplasia. (e) CT images before sacrifice at 4 weeks.

3.3. General evaluation of the ATO-NFCS in the rabbit trachea

In the animal study, the ATO-NFCS was deployed using a 5-F delivery system (figure 4(a)). The process of ATO-NFCS placement in the rabbits is shown in figure 4(b). Stent placement was technically successful in all 18 rabbits. There were no cases of inflammatory cells. To explore the degradation of PLCL in the trachea, the nanofiber membranes of the implanted tracheal stents were retrieved for HE staining (figure 3(e)). Strips of flaky cracks in the PLCL were observed beginning at 1 week poststenting, and splits were observed at 4 weeks after stent placement.
Figure 5. Evaluation of the toxicity and side effects of ATO secondary to ATO-NFCS placement. (a) ALT, AST, TBIL, CREA, BUN and UA levels in blood before stenting and 1 and 7 d after stenting. Almost all the liver function and kidney function indexes in this study remained in the normal range. The range indicated by the horizontal line is the normal range for the corresponding index. (b) Histopathological findings of the heart, liver, spleen, lungs and kidneys in the three groups at 1 and 4 weeks. Images of lung sections from the NFCS group and the 0.4% ATO-NFCS group presented patchy inflammatory cell infiltration at 1 week. Other findings were normal. HE staining, 200x.

pneumothorax or hemoptysis immediately after stent placement in any rabbit. The anatomical results at 4 weeks showed that more newly formed blood vessels were visible on the surface of the trachea in the NFCS group than in the ATO-NFCS groups (figure 4(c)). These results suggest that tracheal inflammation after ATO-NFCS implantation is mild. After tracheal sectioning, the results confirmed that the thickness of the proliferating granulation tissue was slight in the ATO-NFCS groups, which is consistent with the results of chest CT (figures 4(d), (e)).

3.4. Toxicity and side effects of ATO secondary to ATO-NFCS placement

The changes in liver and kidney function are shown in figure 5(a). Compared with before stenting, after stenting, the levels of ALT, AST and TBIL were mildly increased for 1 d in all three groups, but all of these levels remained within their normal range. At 7 d after stenting, the levels of the liver function markers were almost the same as before stenting. The results of multiple comparisons showed a significant difference for only the elevated AST level in the 1.2% ATO-NFCS group 1 d after stenting compared with the PLCL group (P < 0.001). Regarding kidney function, the levels of CREA, BUN and UA for all three kinds of stents were within the normal range at the three time points. Multiple comparisons showed no significant difference (P > 0.05). The pathological findings of the heart, liver, spleen, lungs and kidneys in the three groups at 1 and 4 weeks are shown in figure 5(b). No pathological changes were observed in the heart, liver,
spleen or kidneys. The lungs presented patchy inflammatory cell infiltration in the PLCL group and the 0.4% ATO-NFCS group at 1 week, while the lungs in the 1.2% ATO-NFCS group presented a normal alveolar structure. At 4 weeks, a normal alveolar structure was observed in all three groups. Based on the results of the liver and kidney function assessment and the normal findings of the heart, liver, spleen, lungs and kidneys on pathology, these results demonstrate the good safety of the ATO-NFCS in our research.

3.5. Tracheal pathological evaluation after ATO-NFCS placement

The pathological findings are shown in figure 6(a). The degree of collagen deposition at 1 week in all three groups was mild. The degree of collagen deposition at 4 weeks in the PLCL group was significantly higher than that in the 1.2% ATO-NFCS group (3.67 ± 0.98 vs 2.83 ± 0.72, p < 0.05). The differences between the PLCL and 0.4% ATO-NFCS groups (3.67 ± 0.98 vs 2.92 ± 0.67, p > 0.05) and
the 1.2% ATO-NFCS and 0.4% ATO-NFCS groups (2.83 ± 0.72 vs 2.92 ± 0.67, p > 0.05) were not statistically significant (figure 6(b)). At 1 week, the mean thickness of the submucosa was significantly higher in the PLCL group than in the 1.2% ATO-NFCS group (506.25 ± 153.72 µm vs 309.13 ± 95.35 µm, p < 0.05). The difference between the 0.4% ATO-NFCS and 1.2% ATO-NFCS groups (349.88 ± 96.43 µm vs 309.13 ± 95.35 µm, p > 0.05) was not statistically significant. At 4 weeks, the mean thickness of the submucosa in the 0.4% ATO-NFCS (974.38 ± 264.80 µm vs 650.25 ± 124.02 µm, p < 0.001) and 1.2% ATO-NFCS (974.38 ± 264.80 µm vs 450.75 ± 130.74 µm, p < 0.0001) groups was significantly lower than that in the NFCS group. Meanwhile, there was a significant difference between the 0.4% ATO-NFCS and 1.2% ATO/PLCL groups (650.25 ± 124.02 µm vs 450.75 ± 130.74 µm, p < 0.05) (figure 6(c)).

4. Discussion

The ideal tracheal stent needs to have six characteristics [24, 25]: (i) easy placement and removal; (ii) effective airway expansion; (iii) good histocompatibility; (iv) good adherence to the tracheobronchial wall; (v) no effect on the clearance of sputum; (vi) induction of no or little granulation tissue hyperplasia. In this study, an electrospun nanofiber-covered SEMS eluting ATO was designed and implemented to reduce granulation tissue hyperplasia in the trachea of rabbits. The main findings were as follows: (i) placement of the ATO-NFCS in the trachea through the delivery system under fluoroscopy was feasible; (ii) the ATO-NFCS had good biocompatibility and biological safety; (iii) the ATO-NFCS effectively reduced granulation tissue hyperplasia at 4 weeks poststenting. Thus, placement of the ATO-NFCS in the rabbit trachea is a feasible technique with good biological safety and functionality in inhibiting granulation tissue hyperplasia.

Granulation tissue hyperplasia is a challenging complication after tracheal stent placement, which seriously affect the life quality of patients. The process of granulation tissue formation involves the proliferation of fibroblasts and vascular endothelial cells [26]. Inhibiting the proliferation of fibroblasts is the key point in reducing granulation tissue hyperplasia. The pathways of ATO function include apoptosis induction, differentiation stimulation, and proliferation inhibition [12]. In a study by Yang [27] and colleagues, an ATO-eluting stent reduced neointima formation in a rabbit iliac artery injury model and suppressed in-stent restenosis by reducing the proliferation and inducing the apoptosis of vascular muscle cells. Both in vivo and in vitro studies by Zhao et al [28], have demonstrated that ATO-eluting stent implantation is an effective strategy for rapid re-endothelialization while inhibiting in-stent restenosis. Many in vitro studies have demonstrated the inhibitory effect of ATO on different types of cells, including pulmonary adenocarcinoma Calu-6 and A549 cells, calf pulmonary artery endothelial cells, human umbilical vein endothelial cells, human pulmonary fibroblasts, myofibroblasts and human skin fibroblasts [29–31]. Considering the mechanism of action of ATO against cancer and inflammation, we designed an ATO-eluting tracheal stent and applied ATO in the fabrication of an eluting stent.

The ATO-NFCS has the characteristics of a drug-eluting and covered stent. The nanofiber membrane material is PLCL, which has good biocompatibility and is widely used in the fabrication of bioengineering scaffolds [22]. The results of the cell and tissue compatibility experiments in our study also confirm this performance. The drug release of the majority of drug-eluting stents, especially cardiovascular stents, is examined in PBS solution to simulate the blood environment. However, the trachea is a semicylindrical hollow organ, and the drug release solution consists of mucosal secretions [23]. The ATO-NFCS was expected to release drug on contact with the mucosal secretions and with degradation of the PLCL. In this study, deformation and partial degradation of the nanofiber membrane was observed at 1 and 4 weeks. Meanwhile, degradation of the nanofiber membrane was also observed in tissue at 2 and 12 weeks. To simulate the drug release more realistically, SAF was prepared to simulate stent placement in the tracheal environment. The burst release of ATO was observed, and almost total release was achieved in 1 d. This release could during the inflammation and proliferation phase of wound healing resulting from mechanical injury of the trachea during stent placement [32]. To overcome the shortcomings of sputum retention in silicone stents and ordinary polyurethane-covered metal stents, a hydrophobic surface was developed to accelerate the clearance of sputum from the surface of the nanofiber membrane. This function also has the potential to decrease the rate of bacterial infection in stent placement and the risk of granulation tissue formation [33].

Drug-eluting stents are the most promising type of stent for use in the cardiovascular system. An ATO-eluting cardiovascular stent received a registration certificate issued by the State Food and Drug Administration (Beijing, China) and has since gained postmarketing clinical data [6]. However, no ATO-eluting stents have been applied in nonvascular lumina until now. The primary results of the ATO-NFCS in the rabbit trachea are positive. The thickness of the submucosa and collagen deposition layers in the ATO-NFCS groups were significantly less than that in the NFCS group. The effect of the 1.2% ATO-NFCS was better than that of the 0.4% ATO-NFCS. The inhibitory effect of ATO on granulation tissue formation is similar to that of other drug-eluting stents.
applied in nonvascular lumina [34–36]. But the difference is ATO-NFCS also prevent granulation tissue growth through stent mesh to trachea lumina. The application of paclitaxel drug-eluting tracheal stents in a canine model significantly reduced granulation tissue formation after stent implantation, but granulation tissue still grew through the stent mesh [9]. These results were also observed in a study on sirolimus-eluting biodegradable tracheal stents [37]. However, covered stents have the advantage of preventing granulation tissue from growing through the stent mesh [38]. Thus, the ATO-NFCS applied in the rabbit trachea presented the advantages of both drug-eluting stents and covered stents.

There are several limitations to this study. First, the small sample size of the current study may affect the significance of the findings. However, these results can guide further, more detailed research. Second, the ATO-NFCS was placed in the trachea of normal rabbits, and the wound healing process after SEMS placement in a normal trachea may differ from that in a trachea with benign stenosis. Third, the follow-up duration of 4 weeks represents only the short-term effects. Further research on medium-term and long-term effects is also needed.

5. Conclusion

In conclusion, the present study preliminarily investigated the efficacy of the ATO-NFCS in reducing granulation tissue formation in the rabbit trachea. The results suggest that the ATO-NFCS is safe and easy to place in vivo. The ATO-NFCS is effective for the suppression of granulation tissue formation in the short term.

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