**Slow-Release Drug Deliver System with Polylactic Acid Hydrogels in Prevention of Tracheal Wall Fibroplasia**

**Jinrang Li**, Lili Peng, Jianjun Sun, Hongguang Guo, Kun Guo, Zuogang Li, Yao Tang, Xu Sun, Shuangqing Zhang

**Abstract**

**Objective:** To compare the effect of slow-release mitomycin C (MMC) and dexamethasone sodium phosphate (DSP) to polylactic acid (PLA) hydrogels on the prevention of tracheal wall fibroplasia.

**Methods:** The release speed of MMC and DSP mixed with PLA hydrogels was determined in vitro and in vivo. The rabbit model of tracheal wall fibroplasia was made through scratching the tracheal wall. Forty-two rabbits were divided into 7 groups and 0.2ml PLA hydrogels containing different doses of MMC and DSP mixed with spongia gelatinosa composite were put in semicircular silicone tubes and then were placed on the wound surface of the scratched tracheal wall. An air embolism killed each animal 4 weeks later. The histology of the specimens was studied.

**Results:** The 1mg MMC and 2mg DSP PLA hydrogels could release over 35 days and 28 days respectively in vitro. The tracheal wall fibroplasia in DSP and MMC groups, except the 0.1mg MMC group, was thinner than that in the control group (P<0.05).

**Conclusions:** Both MMC/PLA and DSP/PLA hydrogels can inhibit fibroplasia of the tracheal wall and it can be coated in a Poly L-lactide-co–glycolide (PLGA) scaffold for treating laryngeal-tracheal stenosis in future.

**Key words:** Slow-release drug deliver system, poly lactic acid hydrogels, tracheal wall fibroplasia

**Introduction**

As is known, mitomycin C (MMC) is one kind of medicine that is widely used in clinics for treating tumors, inhibiting fibroplasia formation, reducing the proliferation of granulation tissue, preventing tissue adhesion, and so on. In Ear, Nose and Throat (ENT) clinics, MMC has a certain effect in prevention of laryngeal-tracheal and nasal stenosis [1-4]. However, all the studies are one-time drug administration that use one piece of cotton soaked with MMC (0.4mg/ml) applied locally in the wound surface for 4-5 minutes, and the results show that the problem of adhesion can’t be completely resolved. Vasanth reported that Poly L-lactide-co–glycolide (PLGA) nanoparticles could be used as laryngeal slow-release drug carriers in the murine vocal fold injection model in 2010 [5]. Polylactic acid (PLA) is one kind of injectable and biodegrad-
able material and has good compatibility with biological tissues. Dexamethasone sodium phosphate (DSP) belongs to glucocorticoid medication. It is very cheap and has the function of inhibiting fibroplasia, but with weak adverse reactions for local drug administration. This experiment aims to use PLA as MMC and DSP as a slow-release carrier to study the effect of MMC/PLA and DSP/PLA on inhibiting fibroplasia of trachea in animals. This may also provide an experimental basis for making a Silicone T-tube coated with slow-release drugs in the treatment of laryngeal-tracheal stenosis.

**Materials and methods**

1. **Slow-release condition in vitro**

A dissolved certain amount (4mg) of PLA hydrogels (Daigang Biotechnology Co., Ltd. In Jinan Shandong) with purified water (16ml) (%W/W: 20%), and then put in a 4ºC refrigerated condition. When the mixture turned into a clear solution, we added MMC (Roche Company) and DSP (National Institutes for food and drug control) into the solution, and kept stirring for 10 minutes; we then put MMC/PLA and DSP/PLA solutions at 37ºC for 5 minutes to form gels.

Next, 1ml of 1mg/ml MMC/PLA and 1ml of 2mg/ml DSP/PLA were respectively placed into 10 ml PBS (pH=7.4) in a 15ml tube in the water bath oscillators (SHA-CA) at a constant temperature of 37ºC and speed of 120rpm. 100μL of samples were taken at the 1st hour, 4th hour, 6th hour, 8th hour, 12th hour, 1st day, 3rd day, 5th day, 7th day, 14th day, 21st day, 28th day, and the same amount of PBS was supplemented [6]. All the samples were stored at -80ºC. All specimens were filtered through a 0.45μm biomembrane and then the concentration of MMC and DSP was tested by ultraviolet spectrophotometry at the best UV spectrophotometric absorption values of 365nm and 242nm, respectively. The MMC linearity range of the calibration curves of UV spectrophotometry is \( y = 0.0063x + 0.3745, R^2 = 0.9990 \). The test needed to be repeated 3 times. According to the standard curve, the concentrations of all samples were calculated. The drugs releasing a speed of MMC/PLA and DSP/PLA with time could then be drafted by SPSS 13.0.

2. **In animal vivo study of MMC/PLA and DSP/PLA**

2.1 **Method of drug administration.**

The present study was performed in accordance with the Chinese Public Health Service Policy on Humane Care and Use of Laboratory Animals, as well as the National Institutes of Health “Guide for the Care and Use of Laboratory Animals”. The animal use protocol was approved by the Institutional Animal Care and Use Committee of the Navy General Hospital.

Forty-two New Zealand rabbits aged about 2 months (weight 2.05 ±0.21 kg) were purchased and housed in the Animal Resource Center at the Navy General Hospital. All of the rabbits had free access to food and water. The Institutional Committee for the Care and Use of Animals at the Navy General Hospital approved all of the procedures used in this study. They were randomly divided into 7 groups averagely. From pre-experiment results, 3 different doses of MMC groups (0.1mg, 0.2mg, 0.4mg), 3 different doses of DSP groups (1mg, 2mg and 3mg) and one control group were designed. Sumianxin anesthetized each rabbit with a 1:1 mixture of ketamine (1ml/kg). The animals were then operated on by an incision of the neck to find the trachea of the animals. The outer tracheal wall of the animals was scratched continually until the tracheal wall had significant congestion. Different doses of MMC and DSP were dissolved in PLA solutions, and then the drug PLA solution turned into gels at 37ºC; 0.2ml of drug PLA gels mixed with spongia gelatinosa composite, which were put in semi-circular silicone tubes, were placed on the wound surface respectively in treatment groups, and in the control group there was no drug. An air embolism killed each animal 4 weeks later. All the specimens were fixed with formalin, embedded with paraffin, sliced and stained with H&E, and then observed with a light microscope. The thickness of fibroplasia was calculated by the vertical distances from the highest point of hyperplasia tissue to the outer surface of the tracheal wall.

2.2 **Determination of MMC concentration in plasma**

To study the delivery characteristic of MMC/PLA in vivo and to make sure that the concentration of MMC in plasma is not so high to induce damage, 1ml of blood was drawn from
the ear vein every time on each rabbit on the 1st day, 3rd day, 7th day, 14th day, 21st day, and 28th day after operation in the 0.4mg MMC group. All the blood samples were centrifuged at 4ºC 3000rpm for 15mins, and then supernatant plasma was taken and stored at -80ºC. 200μl of rabbit plasma and 10μl internal standard of triamcinolone (TCL) (National Institutes for food and drug control) were mixed in a 4.0ml eppendorf tube, and then 2.2ml organic solvents were added and were kept shaking for 2 minutes. The mixture was centrifuged at a high speed of 6000rpm at 4ºC for 5 minutes, and then 2.0 ml of supernatant plasma was drawn, centrifuged, concentrated and evaporated at 40ºC (Nitrogen evaporator: N-EVAP112), and 200μl mobile phase was added to supernatant plasma for dissolving; the plasma was then kept shaking for 1 minute and centrifuged at 7000rpm at 4ºC for 5 minutes. 10μl of treated supernatant plasma was precisely drawn for sample analysis. The concentration of plasma of all the specimens was detected by mass spectrometry [Triple quadrupole tandem mass spectrometer (Thermo TSQ Quantum Access), high liquid chromatography (Thermo Accela)]. The linearity range of the calibration curve is $y= 0.000847+0.0094x$, and $R^2 = 0.9955$.

Figure 1. DSP cumulative concentration in vitro

2.3 Routine blood test

1.5ml of blood was drawn from the ear vein every time on each rabbit on the 1st day, 7th day, and 14th day after operation and was then sent for a routine blood test in an hour.

3. Data analysis. All data were analyzed through Statistical Package for the Social Sciences (SPSS) software, version 13.0. Quantitative data, which concerned approximate normal distribution, were described by mean±SD; a comparison in multiple group samples was tested by one-way analysis of variance (ANOVA). Fisher’s least significant difference test was used for significant analyses of variance.

Results

1. In vitro MMC/PLA & DSP/PLA delivery

1ml of PLA hydrogels that contained 1mg MMC and 2mg DSP could release over 35 days and 28 days, respectively. DSP/PLA released fast in the first 6 hours; the cumulative concentration achieved 31.51%±2.31%, and 92.01%±1.76% on the 28th day; the speed of drug delivery then became slow (Figure. 1). MMC/PLA released very slowly in the first 3 weeks; the cumulative concentration of the 21st day was 32.05%±1.81%, and 84.28%±3.06% on the 35th day; from then on the speed of delivery became faster (Figure. 2), maybe due to the instability of MMC.

Figure 2. MMC cumulative concentration in vitro

2. In vivo MMC/PLA delivery

The plasma concentration of the 0.4mg MMC group was determined by ultra-high-performance liquid chromatography - electrospray ionization - tandem triple quadrupole mass spectrometry. The drug of the plasma concentration achieved a peak on the 7th day (Figure. 3), and tended to remain stable after 10 days. The concentration of MMC in plasma was very low. We had a routine blood test on the 1st day,
7th day, and 14th day after operation. The results of the white blood cells of the 1st day were \((9.03\pm1.57)\times10^9/L\); the 7th day results were \((9.85\pm1.63)\times10^9/L\), and the 14th day results were \((10.17\pm3.11)\times10^9/L\). There were no significant differences of white blood cells among 3 different times \((P>0.05)\). That means that MMC of the 0.4mg dose didn’t have any influence on white blood cells. Owing to mild adverse reactions of low doses of DSP for local administration, the concentration of DSP was not detected.

**Figure 3.** The mean MMC plasma concentration from 1st day to 21st day in 0.04mg MMC group

**Table 1** Comparison of the thickness of fibroplasia between control group and other different dose groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thickness of fibroplasia (mm)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.82±0.29</td>
</tr>
<tr>
<td>0.1mg MMC</td>
<td>0.65±0.16</td>
</tr>
<tr>
<td>0.2mg MMC</td>
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</tr>
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* Fisher’s least significant difference test, \(P<0.05\)

3. **The thickness of fibroplasia with H&E staining**

MMC/PLA and DSP/PLA hadn’t completely degraded after 4 weeks. The thickness of tracheal wall fibroplasia in the control group \((0.82\pm0.29\text{mm})\) had significant differences, compared to all the different dose groups \((p<0.05)\), except the 0.1mg MMC group \((0.65\pm0.16\text{mm})\), which means the 0.1mg MMC hadn’t achieved a treating dose (Table 1). Among MMC groups, the 0.4mg MMC group \((0.17\pm0.09\text{mm})\) had differences in reducing granulation tissue formation from other dose groups \((p<0.05)\) (Table 2); the 0.4mg MMC group could best inhibit fibroplasia in this experiment (Figure 4, 5, 6, 7). Among DSP groups, the differences of the thickness of fibroplasia were not significant \((p>0.05)\) (Table 3); 1mg of DSP was the most appropriate dose to treat fibroplasia in rabbits (Figure 8, 9). The 0.4mg MMC group and 3 different doses of DSP groups had no significant difference in inhibiting fibroplasia \((p<0.05)\) (Table 3).
Figure 6. Fibroplasia of the tracheal wall in 0.2mg MMC Group (HE×40)

Figure 7. Fibroplasia of the tracheal wall in 0.4mg MMC Group (HE×40)

Figure 8. Fibroplasia of the tracheal wall in 1mg DSP Group (HE×40)

Figure 9. Fibroplasia of the tracheal wall in 2mg DSP Group (HE×40)

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* Fisher’s least significant difference test, P>0.05

Discussion

MMC was first discovered in 1956, which was one kind of broad-spectrum antineoplastic antibiotics separated and extracted from head-like streptomyces culture solution; its mechanism is depolymerizing DNA in cells, impeding reproduction, thereby inhibiting cell mitosis (including tumor cells and fibroblast). MMC can inhibit cell division, protein synthesis, and fibroblast proliferation. As a broad-spectrum anticancer drug, MMC is used as systemic chemotherapy drugs,
such as in breast, lung, pancreas and colorectal cancer treatment. Rabbar’s report shows the efficacy and safety of MMC as an adjuvant treatment in the management of selected cases of laryngeal and tracheal stenosis [7]. DSP, as one kind of glucocorticoid, has a significant effect on inflammation, which can be caused by various factors, such as Physics, Chemistry, Biology, Immunology and so on. During the early time of inflammation, it can relieve leakage, edema, capillary expansion, leukocyte infiltration, and a phagocytic reaction. In the late time, it can inhibit hyperplasia of capillary and fibroblasts, delay granulation tissue forming, prevent adhesion and scar formation. Fang’s research indicates that flumeprednisolone and spongia gelatinosa composite is effective in preventing peridural adhesion after an intervertebral disc operation [8], but studies about local application of DSP for treating scar formation haven’t been reported.

Temperature-sensitive PLA hydrogel, which is biodegradable and has good biocompatibility with tissue, can change with ambient temperature into a reversible phase [9]. Taking advantage of the PLA phase-change characteristic, it can be served as filling material as a result of a tissue defect or drug slow-release carrier. PLA hydrogel is liquid in a refrigerated condition and colloid at room temperature, which can be applied in laryngeal-tracheal diseases as a drug delivery carrier. When temperature-sensitive PLA gel is injected into the body, it will be curdled in situ at body temperature. We can mix the required doses of drugs with PLA gel, which can be injected into disease-affected sites and which can form gel in a short time. These in-situ formed hydrogels, which can be fixed in certain parts of the body, have a good solid combination with the surrounding tissue; it can precisely release wrapped drugs in lesions and effectively control the extent of drug delivery, thereby reducing systemic adverse reactions.

Regardless of whether we use in-vivo or in-vitro drug-release experiments, we find that PLA gel wrapped with drug release slowly. A dose of 2mg DSP with PLA can sustainably release up to 4 weeks in vitro, and 1mg MMC with PLA can deliver over 5 weeks. MMC/PLA of the 0.4mg group has not released completely after 21 days in rabbits; the peak concentration achieves in blood on the 7th day; it releases slowly and has weak adverse reactions on the body, which can be served as a good carrier of antineoplastic, and has a long-lasting effect on the tumor.

MMC has been successfully used in the suppression of scar formation, particularly in the field of ophthalmology [10-11]. This is due to its modulation of fibroblast activity, which enables decreased scarring and fibrosis. In otolaryngology head and neck surgery clinics, the use of MMC is more and more common. From this study we know that, in regards to the inhibition effect of scar formation, both MMC and DSP can successfully reduce granulation tissue with different doses; the 0.1mg MMC group hasn’t achieved enough of a dose to inhibit fibroplasia, compared to the control group; in the 0.4mg MMC group, we can see that it has the least fibrous tissue. Among doses of the 1mg, 2mg and 3mg DSP groups, the effect of suppressing fibroplasia isn’t statistically significant either; we can therefore get a conclusion that 1mg of DSP is the most appropriate dose in inhibiting fibroplasia on the tracheal wall in this research, and comparing different doses of DSP groups to the 0.4mg MMC group, which gets the best treatment among MMC groups, results of treating fibroplasia are nearly the same. Both MMC and DSP can be used in ENT clinics for preventing laryngeal-tracheal and nasal stenosis. The sustained releasing drug delivery system has superiority to traditional one-time usage of MMC [12].

With the development of molecular biological materials and the improvement of fabrication technology, the tissue compatibility is getting better; therefore, its clinical use is extended and much closer to the clinical application. In recent years, more and more researches about PLA have been studied and clinical application has been promoted; its biological property has been better understood. In this study, we use PLA as a sustained-release carrier for local administration in the animal model to prevent proliferation of fibrous tissue of the tracheal wall. Laryngotracheal stenosis troubles ENT doctors a lot; the main reason for laryngeal-tracheal stenosis is trauma, which often has fibroplasia and which forms stricture in airways. Coronary drug-eluting stents are widely researched and applied in cardiovascular clinics [13-14]. Laaksovirta reported using PLGA (Poly L-lactide-co–glycolide) scaffolds as a urethra prostatic spiral stent to relieve urinary tract obstruction in 2001 [15]. Robey’s experiment shows that biodegradable PLGA stents can be used internally to stabilize and support surgically reconstructed airways [16]. PLGA and PLA maintain a certain shape in a certain period of time. Depending on different ratios of the polylactic acid and polyglycolic acid, different degradation rates, hardness and strength of stents can be developed that can wrap MMC and DSP for preventing granulation tissue and fibroplasia. In the early study, we planned on establishing an animal model of tracheal stenosis, but New Zealand rabbits can’t survive after a tracheo-
otomy because of their fine trachea. We therefore changed the animal model to fibroplasia of the tracheal wall in rabbits and it was successfully established. We found that the PLA-wrapped MMC and DSP can prevent granulation tissue and fibroplasia of the tracheal wall. Thus, in the future, we can invent a PLGA scaffold coated with MMC or DSP for sustainably delivering. This kind of scaffold can not only maintain the airway lumen and biodegrade at last without removing but can also inhibit fibrous formation use in the treatment of laryngeal-tracheal stenosis. It needs further study in big animals, such as dogs, to prove the hypothesis.

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**Conflict of interest statement**

The authors do not declare any conflict of interest or financial support in this study.

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