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Local, combination chemotherapy in prevention of cervical cancer recurrence after surgery by using nanofibers co-loaded with cisplatin and curcumin

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Locoregional recurrence of cervical cancer following surgical resection remains a severe clinical problem. To reduce tumor replase rate with more efficacy and safety, local combination chemotherapy may has its advantage over mono-chemotherapy or systemic chemotherapy. The aim of this study was to evaluate the efficacy of electrospun nanofibers co-loaded with cisplatin and curcumin to prevent local recurrence of cervical cancer after surgery. *In vitro* tests, the combination of cisplatin and curcumin achieved a synergetic effect in growth inhibition and apoptosis induction of Hela cells. *In vivo* trails, local implantation of nanofibers enabled both drugs to be highly accumulated at the surgical site with an optimum concentration ratio between the two drugs. When used in the prevention of U14 cervical cancer recurrence in mice, nanofibers-based local combination chemotherapy was more effective and less toxic than systemic combination chemotherapy, indicating its great clinical potential in the future.

Introduction

Although radical hysterectomy with pelvic lymphadenectomy has been widely accepted as the treatment in patients with cervical cancer, 10-20% of the patients recurred after radical surgery, and five-year survival rate is less than 5%. To reduce recurrences and improve survival in patients with high-risk factors, concurrent chemoradiation(CTRT) has long been held as the optimum postoperative adjuvant therapy.[1,2]

As far as chemotherapy is concerned, cisplatin (abbr. as cis) has long been the first-line chemotherapy drug against cervical cancer by systemic administration as an adjuvant to radiotherapy.[3] However, it has been widely recognized that cisplatin-based combinations have significant toxicity and usually lead to many complications and side effects.[4]

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In recent years, curcumin (abbr. as cur), an extract from the rhizome of curcuma longa, has aroused wide concern as the adjunct drug to cancer chemotherapy for the purpose of improving the efficacy, reducing the risk of drug resistance, and extenuating the drug toxicity. With these advantages, however, drawbacks associated with light sensitivity, low water solubility, and poor oral bioavailability restrict the therapeutic value of curcuminoids. To improve the curcuminoid solubility, stability, and bioavailability, various nanocarriers have been investigated, such as liposomes, polymeric nanoparticles, microspheres, cyclodextrin, hydrogels and nanofibers.[5-13]

Although many studies have successfully achieved the co-delivery of curcumin and various chemotherapeutics (i.e. paclitaxel, doxorubicin,) in a single formulation, it is no easy job to achieve the co-delivery of curcumin and platinum drugs which are usually difficult to be encapsulated in the nanocarriers.[14-17]

Same like combination chemotherapy in pursuit of better efficacy and lower systemic toxicity, polymer delivery vehicles for implantation intra-tumorally or adjacent to the cancerous tissue has been studied extensively as a means of achieving high therapeutic concentrations of chemotherapy to the site of malignant disease and avoiding the entrance of drug into the blood circulation and other normal organs., such as gels, nanoparticles, polymeric films, rots, wafers, nanofibers and so on. Biodegradable devices disappear following complete release of the loaded active agent, eliminating the need for a second surgery for device removal and the risk of chronic foreignbody immune response.[18-21]

Based on the discussions above, it is reasonable to believe that

polymer-based, combinational, local chemotherapy will play an important role in the prevention of local tumor recurrence. To address the need for both co-delivery of cisplatin and curcumin in one carrier, and for local chemotherapy, and for simple preparation process, electrospun nanofibers were used in the present study to simultaneously deliver cisplatin and curcumin. As well known, the high encapsulation efficiency, high loading capacity, simultaneous delivery of diverse therapies, ease of operation, and cost-effectiveness are appealing features for electrospinning used in drug delivery.[22-24] The use of electrospun fibers as drug carriers is promising in future biomedical applications, especially postoperative local chemotherapy. The surgeons can conveniently place the drug-loaded fibrous mat on the surface of the wound after the removal of the solid tumor to prevent the local tumor recurrence.[25-27] When applied in the treatment of cervical cancer, repeated drug administration can be achieved by vaginal implantation of nanofibers mat which can be conveniently adjusted in shape and size to fit the geometries of the vagina or cervix. In our previous study, the efficacy of cisplatin-loaded poly (ethylene oxide)/polylactide (PEO/PLA) composite electrospun nanofibers were used as a local chemotherapy system against cervical cancer in mice *via* vaginal implantation. The nanofibers were proven to have good mucoadhesive property by *in vitro* mucoadhesion test and *in vivo* vaginal retention evaluation due to the significantly improved hydrophilicity by the addition of PEO. The *in vivo* anti-tumor trials showed that a better balance between efficacy and systemic safety was achieved by nanofibers implantation than that by i.v injection due to local accumulation of cisplatin released from the nanofibers in the vaginal tract.[28]

Therefore, in the present study, nanofibers-based, local, combination chemotherapy was investigated to prevent the local cervical cancer recurrence after surgery. PLA/PEO nanofibers simultaneously loaded with cisplatin and curcumin (abbr. as cis-cur/fiber) were prepared by electrospining. Then we tried to prove the advantage of combination chemotherapy over mono-chemotherapy *in vitro* test, and the superiority of nanofibers-based, local, combination chemotherapy to systemic, combination chemotherapy *in vivo* trails.

Results and discussion

Combined effect of cisplatin and curcumin on HeLa cell viability

To investigate the combined effect of cisplatin and curcumin on the cell viability of HeLa cells, the cells were treated with cisplatin (6.0μmol/l), curcumin (10, 20, 40 and 80μmol/l) or their combination for 48 h, respectively. The inhibition rate was determined by Methyl thiazolyl tetrazolium (MTT) assay. As seen in Fig.1A, curcumin inhibited cells proliferation in a dose-dependent manner. When low dose of curcumin (10 or 20μmol/l) was combined with cisplatin (6.0μmol/l), the combination suppressed the proliferation of HeLa cells more effectively than each of them used alone, but it is not the case when high dose of curcumin (40 or 80μmol/l) applied. A maximum synergistic interaction (cells inhibition rate: 0.76±0.028; q=1.214031, q>1.15) occurred when 6 μmol/l of cisplatin and 20 μmol/l of curcumin were used in combination (the concentration ratio between curcumin and cisplatin was 3.33) (Fig.1B). Then the ratio of 6:20 was selected for apotosis study.

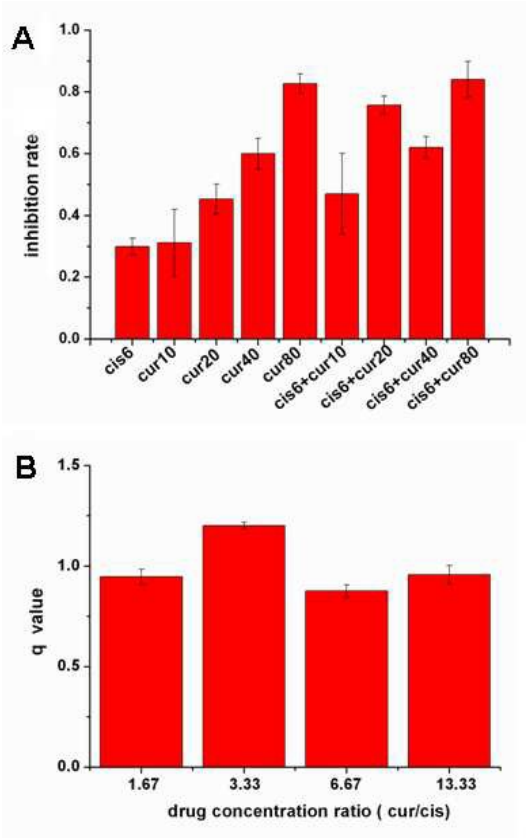

Fig. 1 (A) Inhibitory effect of cisplatin or curcumin or their combination on proliferation of HeLa cells: (B) Q value of combination groups. The results were given as mean value ± SD, over three MTT tests.

Combined effect of cisplatin and curcumin on HeLa cell apoptosis

To investigate further, the apoptosis-inducing effect of cisplatin, curcumin and their combination was evaluated by Annexin V/PI staining. HeLa cells were treated with 6 μmol/l cisplatin, 20 μmol/l curcumin or their combination for 20 h, followed by flow cytometry analysis. As shown in Fig.2A and 2B, the percentage of late apoptosis in the blank cells, cells treated with cisplatin or curcumin was detected to be 0.1%, 3.3 and 37.8%, respectively. In line with the findings of the MTT assay, the late apoptotic cells rate of the combination chemotherapy was significantly increased to 62.7% compared with the monotherapy. The combination chemotherapy markedly enhanced apoptosis of HeLa cells, which indicates that the application of curcumin significantly increased the effectiveness of cisplatin.

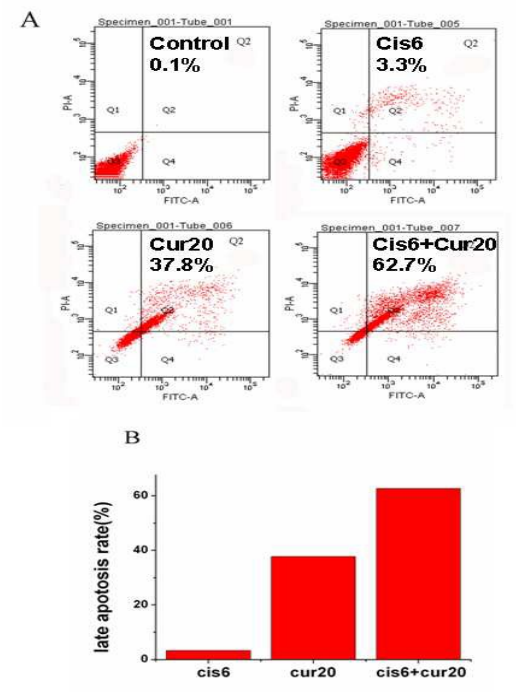

Fig.2 Combined effect of cisplatin and curcumin on Hela cells apoptosis. (A) Annexin V/PI apoptosis analysis of Hela cells after different treatment. (B) Summaries of the late apoptosis rate in histograms.

Characterization of cis-cur/fiber

Consistent with our previous study, the composite PEO/PLA was used to prepare nanofibers co-loaded with cisplatin and curcumin by electrospining[28]. For the purpose of preventing cervical cancer recurrence after surgery, the enhanced hydrophilicity of nanofibers mat by the addition of PEO (one third the mass of PLA) was the key factor to its mucoadhevisity which is crucial to vaginal delivery. As an indication of wetting, the contact angles are correlated to the strength of mucoadhesiveness. The wettability of cis-cur/fiber was characterized by dynamic contact-angle measurement. The initial water contact angles of the composite fiber-mat were 117.4±1. 6°and rapidly decreased to 47±5. 3°within 10s, then fell to 0°within 20s(Fig.3A).

ESEM micrographs of cis-cur/fiber revealed the morphology of nanofibers. As shown in Fig.3B, the nanofibers were uniform in diameter (588 - 703 nm) and smooth in appearance without drug crystals, indicating that slightly water-dissolved cisplatin and hydrophobic curcumin had been successfully encapsulated in fibers.

Since curcumin is a fluorescent molecule, to gain information on the distribution of curcumin in the fibers, confocal laser scanning microscope (CLSM) observations were carried, showing that curcumin was uniformly distributed in the nanofiber(Fig.3C).

The content of curcumin and cisplatin in the cis-cur/fiber were analysied by inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC), respectively. The results showed that 14.5±2.3% of curcumin and 3.0±0.4% of cisplatin in weight percent of polymers used was loaded in the nanofibers mat, with the encapsulation efficiency being 87.0% for curcumin and 60% for cisplatin, respectively.

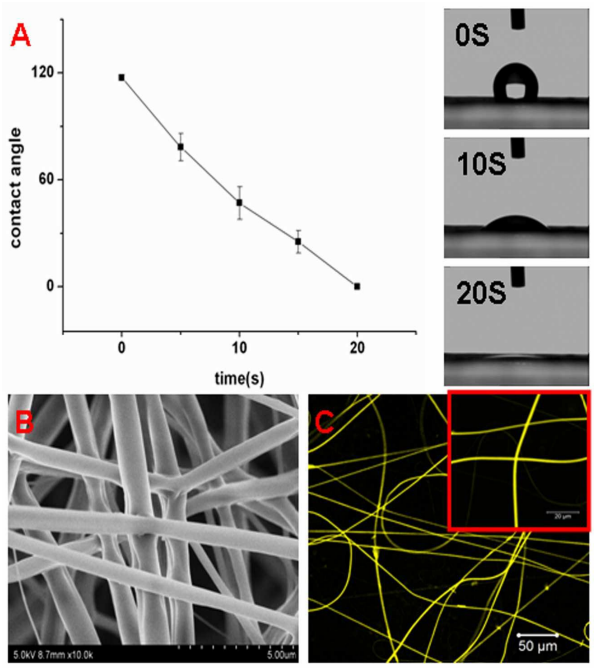

Fig. 3 (A) Curves of soaking speed of cis-cur/fiber. Each data point represents the average of triplicate samples and error bars represent standard deviation (n=3); (B) ESEM images of the electrospun cis-cur/fiber. Bar=5μm; (C) confocal images of the cis-cur/fiber, bar=50μm; inlet bar=20μm.

***In vitro* drug release of cis-cur/fiber**

As multiple drugs were incorporated in the nanofibers, the release behaviors of both cisplatin and curcumin from this system were investigated in the release medium with or without the emulsifier. Tween-80 was chosen as the emulsifier to maximize the bioaccessibility of curcumin *in vitro*. As shown in Fig.4, in the PBS (pH 7.4) with Tween-80, a quick release of cisplatin took place at the beginning with 78.4% of cisplatin released from the nanofibers within half an hour. In contrast, the release kinetics of curcumin could be illustrated in two stages: around 32.8 % of initial burst release of curcumin from cis-cur/fiber at 10 h, mainly because of the diffusion of drugs near fiber surfaces. After 10 h, the release of curcumin began to level off. In the medium without Tween-80, initial burst release of cisplatin at 10 h decreased to 47.3% whereas the minimum release of curcumin was observed with only 2.1% released at 72h.

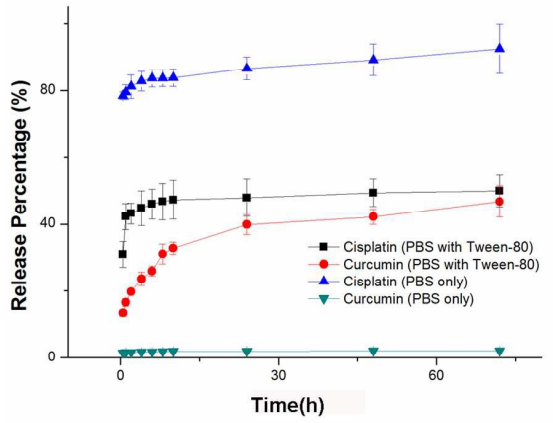

Fig.4 Release behaviors of cisplatin and curcumin from cis-cur/fiber *in vitro*.

Biodistribution of cisplatin and curcumin

The biodistribution of cisplatin and curcumin was studied by ICP-MS and HPLC, respectively. The same dose of the two drugs was administrated by the two distinct administration routes- subcutaneous fiber implantation in the armpit and systemic injection (i.v or i.p), leading to different drug distribution profiles. As shown in Fig.5, several features of biodistribution were summarized as follows:

(1) Both cisplatin and curcumin released from the cis-cur/fiber mainly accumulated in the targeted armpit tissue. At 1h, 10h and 24h after drug administration, the concentration of curcumin in armpit released from the cis-cur/fiber was as high as 17.3±3.5, 28.9±5.2 and 21.0±4.6 μg/g, respectively, whereas only 7.7±1.6, 6.5±1.4 and 6.3±1.0 μg/g in cis-cur/i.v-i.p group, respectively (Fig.5A). And the concentration of cisplatin in armpit released from the cis-cur/fiber was around 2.8±1.0, 11.6±1.9 and 9.4±2.0 μg/g, respectively, compared with 1.3±0.3, 1.2±0.2 and 0.4±0.1 μg/g in cis-cur/i.v-i.p group, respectively (Fig.5D). The ratio of cisplatin concentration in the armpit between fiber implantation and systemic injection at 1h, 10h and 24h was 2.3, 7.4 and 8.2, respectively, while 2.2, 4.5 and 3.3 in the case of curcumin. (Fig.5G).As expected, two drugs were simultaneously delivered to the targeted site and significant local drug accumulation was achieved by nanofibers implantation, indicating that electrospun nanofibers are competent to local combination chemotherapy.

(2) Local drug delivery by nanofibers is favorable to reduce the drug distribution in important metabolic organs such as liver and kidneys. For example, the curcumin concentration at 10h after injection was 45.6±8.8 μg/g in liver (Fig. 5B) and 15.0±2.5 μg/g in kidney (Fig. 5C), which was 2.4 and 0.89 times that by fiber implantation, respectively. While the cisplatin concentration at 10h after injection was 6.1±1.0 μg/g in liver(Fig.5E) and 8.5±0.9 μg/g in kidney (Fig. 5F), which was 2.1 and 1.6 times that by fiber implantation, respectively. Comparatively, there was no much difference of drug concentration in the kidneys between systemic injection and fiber implantation for curcumin, possibly due to the fact that hydrophobic curcumin is readily to accumulate in the liver rather than kidneys after systemic injection[29]. The reduced drug concentration in liver and kidneys would undoubtedly be favorable for the systemic safety of the fibrous mats.

(3) At 10h after fiber implantation, the concentration ratio between curcumin and cisplatin dropped to 3.1 (Fig.5H), a ratio which probably allows the optimum synergetic effect to kill the residual tumor cells according to the results of cells experiment mentioned above(q=1.21 when ratio is 3.33). It still needs further studies to explore whether the concentration ratio around 3.33 will achieve the greatest synergetic effect *in vivo* just like *in vitro* test. If true, this kind of synergetic effect will possibly lead to effective elimination of residual tumor cells.

***In vivo* treatment of cervical cancer**

The *in vivo* antitumor efficacy of cis-cur/fiber was studied in female KM mice bearing subcutaneous cervical cancer. Because of the limitations of experimental conditions, we established the tumor recurrence model in KM mice by using murine U14 cell lines instead of human-derived HeLa cells and corresponding nude mice.

After resection of the primary tumor, mice were randomized to three treatment groups: implantation of cis-cur/fiber (group 1), injection of cisplatin and curcumin(group 2), tumor resection only(group 3). Then the efficacy and safety of different therapies were compared.

As shown in Fig.6A, local tumor recurrence was found in 71.4% animals within 4 days after tumor resection in group3 while 28.6% and 0% in group2 and group1. At day 22 post-surgery, the recurrence rates were 25%, 80% and 85.7% in group1, 2 and 3, respectively.

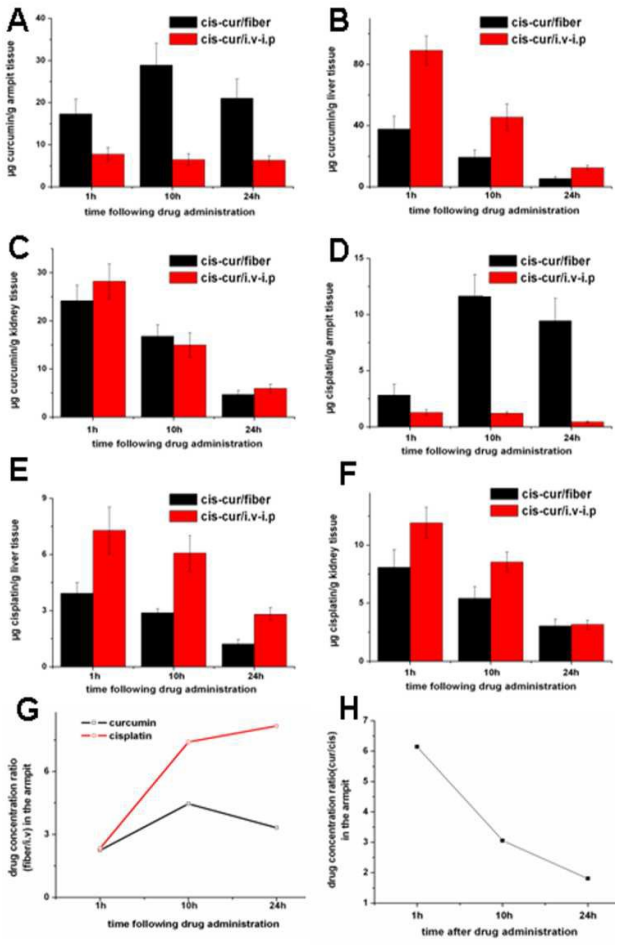

Fig. 5 Biodistribution of cisplatin and curcumin at 1, 10, and 24h after cis-cur/fiber implantation or systemic injection. (A-C) represent the curcumin concentration in armpit, liver and kidney tissue, and (D-F) represent the cisplatin concentration in armpit, liver and kidney tissue, respectively; (G) the drug concentration ratio in the armpit between fiber implantation and systemic injection; (H) the drug concentration ratio in the armpit between curcumin and cisplatin after cis-cur/fiber implantation. The results were given as mean value ± SD, over two mice in a group.

Fig.6B showed that during the initial period after tumor resection, the growth of recurrent tumor was significantly inhibited by cis-cur/fiber implantation while the average tumor volume in control group increased rapidly. The combination chemotherapy by i.v(cis) or i.p(cur) failed to suppress the proliferation of tumor cells, especially in the late period of experiment possibly because the relatively rapid clearance of the drug from the body.

More seriously, systemic combination chemotherapy led to great toxicity during the initial days after drug injection. Average weight losses of 10% were observed on day 4 in the mice injected with cisplatin(0.15 mg) and curcumin(0.5mg), resulting in the death of two in total seven mice within a short period of nine days after drug administration. In sharp contrast, the body weight of the mice in fiber implantation group and control group basically kept a steady rising trend and the relative body weight (wt/w0) was always above 1 (Fig.6C), suggesting that nanofiber-based local chemotherapy is much safer than systemic chemotherapy.

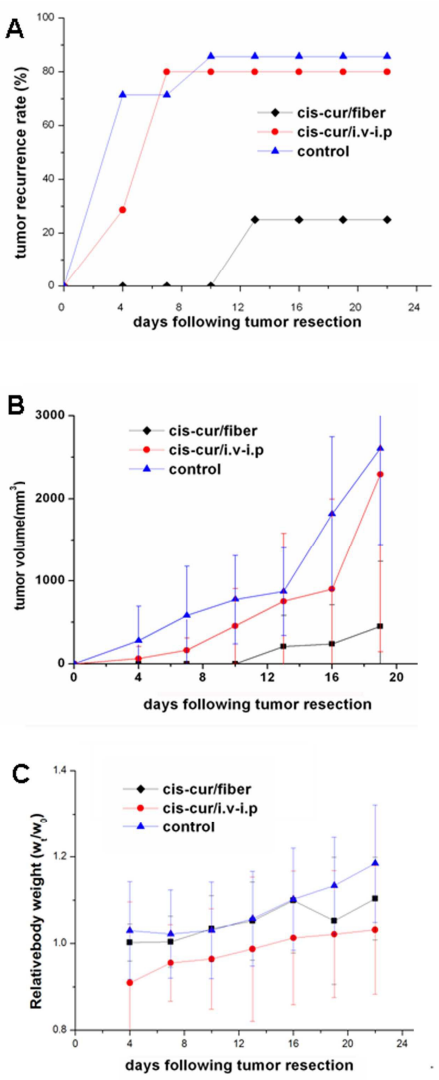

Fig.6 Tumor recurrence rate (A), tumor volume (B) and relative body weight(C) of mice receiving different treatment after U14 subcutaneous tumor resection.

As shown in Fig. 7A, histological analysis showed that a large area of degeneration or necrosis was induced by cis-cur/fiber and few tumor nests were observed on the 4th day after tumor resection, whereas large amounts of viable tumor cells spread over the armpit tissue in cis-cur/i.v-i.p (Fig. 7B) and control group (Fig. 7C), suggesting that cis-cur/fiber can more effectively kill the remnant tumor cells during the initial period following the surgery compared with systemic injection.

At day 14 post-surgery, a representative mouse from each group was taken picture to show the visible morphology. In cis-cur/fiber group, six in eight (6/8) mice didn't show any obvious sign of tumor recurrence and their wounds healed well as shown in Fig.7D, whereas visible tumor nodule could be observed in the original surgical site in 80% of mice (4/5) in cis-cur/i.v-i.p group (Fig.7E) and 85.7% of mice (6/7) in control group (Fig.7F).

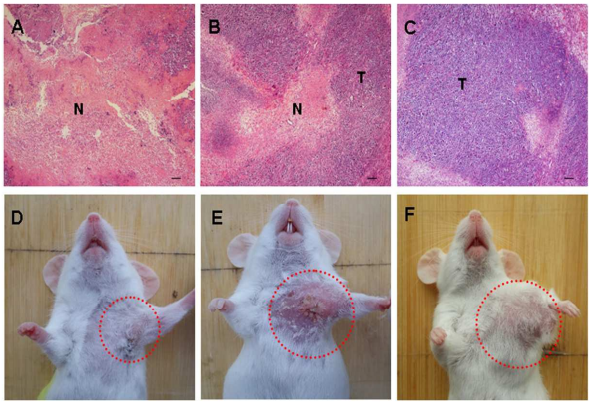

Fig.7 (A-C) showed histopathological observation of armpit/tumor tissue in cis-cur/fiber group (A), cis-cur/i.v-i.p group(B) and control group(C) on the 4th day after tumor resection. N = necrosis or degeneration; T = viable tumor tissue, bar = 500 μm. (D-F) showed macroscopic observation of tumor recurrence and wound healing on the 14th day after tumor resection in cis-cur/fiber group (D), cis-cur/i.v-i.p group(E) and control group(F).

According to the results of *in vivo* trails, it is concluded that local, combination chemotherapy by nanofibers implantation is more effective and safer than systemic combination chemotherapy, undoubtedly owing to its local, sustained drug distribution profile.

Conclusions

The combination of cisplatin and curcumin was proved to be more effective against Hela cells than each used alone *in vitro* tests. PLA/PEO nanofibers simultaneously loaded with cisplatin and curcumin was prepared. *In vivo* trials showed that the cis-cur/fiber led to preferred partition of cisplatin and curcumin in surgical site much more than that by vein injection. The optimum concentration ratio between the two drugs at the targeted site was achieved about 10 hours after the fiber implantation, possibly producing the greatest efficacy against the residual tumor cells. Compared to combination, systemic chemotherapy, nanofibers-based combination, local chemotherapy is more successful in the prevention of local cervical tumor recurrence from the aspects of lower tumor recurrence rate, suppressed tumor growth and less body weight loss. In short, nanofibers mat is a hopeful dosage form for local, combination chemotherapy as an auxiliary option to prevent local cervical cancer recurrence after surgical resection.

Experimental

Materials PLA was synthesized in our laboratory. Its molecular weight (Mn) and polydispersity (PD) determined by GPC were 138,000 and 3.41, respectively. PEO (Mw = 100 kg mol^{-1}) was purchased from Alfa Aesar Co. Ltd. (Tianjing, China). Cisplatin was purchased from Hisun Pharmaceutical Co. Ltd. (Zhejiang, China). Curcumin was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd.

Cell cultures HeLa cells (human cervical carcinoma) were kindly supplied by Changchun Institute of Applied Chemistry. HeLa cells were maintained in DMEM medium, supplemented with 10% FBS (Gibco BRL, Rockville, MD), 100U/L of streptomycin and 100 IU/mL of penicillin (Sigma, St. Louis, MO). The cell cultures were incubated in a humidified atmosphere of 5% CO_2 at 37℃. Mouse uterine cervical cancer U14 cell line was purchased from the Medical Department of Jilin University in China, cultured and passaged in the form of ascites in the abdominal cavity of the mice.

MTT assay The cytotoxicity of cisplatin, curcumin and their combination against HeLa cells was measured by the MTT assay *in vitro*. Briefly, HeLa cells were seeded in 96-well plates at a cellular density of 5×10^3 cells per well and incubated overnight at 37℃, and then treated with 6.0μmol/l cisplatin, 10, 20 , 40 and 80μmol/l curcumin or their combination, respectively. After 48 hours, 20μl of MTT solution was added to each well and the cells were incubated for 4 hours. The medium was aspirated and replaced with 150 μl DMSO to solubilize the formazan crystals. The plates were agitated for 10 minutes and the optical density (OD) at 490 nm was measured using a microplate reader. The inhibition rate of cell proliferation was calculated: inhibition rate (%) = (OD value of control group – OD value of experimental group) / OD value of control group) x 100%. The q formula was used to evaluate the interaction of mixtures, and synergism or additivity was obtained. Q value = EAB / (EA + EB – EA×EB). In the formula, EAB was the inhibition rate of the combination of cisplatin and curcumin. EA and EB were the single drug inhibition rate. If q>1.15, the actual effect was synergism, if $0.85 \le q \le 1.15$, the actual effects was additivity, and if $q < 0.85$, the two drugs might be mutual antagonism.

Annexin V/PI flow cytometric analysis The apoptotic rates of HeLa cells after treatment were determined by flow cytometry using an Annexin V-FITC / PI apoptosis kit (KeyGEN Biotech, China). HeLa cells were seeded in 6-well plates at a density of $2x10^6$ per well overnight, and then treated with 6.0μmol/l cisplatin, 20μmol/l curcumin or their combination for 20 h. The cells were harvested and washed twice with PBS. After staining according to the manufacturer's instructions, Cell cycle was analyzed by EPICS-XL flow cytometer (Beckman Coulter, USA) and data were analyzed by Mcycle software.

Preparation and characterization of the cis-cur/fiber PEO was dissolved at 10% (wt/vol) trifluoroethanol (TFE), followed by the addition of Cis at 5% and Cur at16.67% in weight percent of polymers used. Then the mixture was added dropwise into the blend solution of PLA and $CHCl_3$ under high-speed stirring, until we got a homogeneous viscous solution. The weight ratio of PEO and PLA was 1:3. Electrospinning parameters were as follows: electric field strength: 1.8-2.0 kV·cm^{-1}; air gap distance: 12 cm; inner diameter of spinneret: 0.4 mm; flow rate of solution: 1-2 ml/h and all the experiments were conducted at room temperature in air.

The morphology of the cis-cur/fiber was determined using an environmental scanning electron microscope (ESEM, Model XL 30 ESEM FEG from Micro FEI Philips).

A confocal laser scanning microscope (LSM 780, Carl Zeiss Inc., Jena, Germany) was used to evaluate the presence and distribution of curcumin in the electrospun composite nanofibers.

Water contact angles of the cis-cur/fiber were monitored with a video contact-angle instrument (DSA100 Kruss GmbH, German) to assess the wettability of the fiber-mat, As 2 μl of deionized water was automatically dropped onto the fiber-mat, the contact angle was determined within 20 sec.

To further confirm the homogenous dispersion of cisplatin or curcumin in the nanofibers, four samples were taken from different parts in the nanofiber mat and the drug contents were determined by inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC), respectively.

***In vitro* release** Release fluid was obtained by phosphate buffered saline (PBS, pH 7.4) with 1% Tween-80 or PBS only. The Cis-cur/fiber (1×1 cm^2) were incubated in 8 ml of the release fluid at 37℃ in a thermostated shaker with a shaking speed at 100 cycles min^{-1}. At pre-set intervals, 1ml of the released solution was collected and the same amount of release fluid was added back. The concentration of cisplatin and curcumin in the sample solutions was determined using the inductively coupled plasma mass spectrometry (ICP-MS) and UV-Vis spectrophotometer.

Animals Female Kunming (KM) mice (30-40 g, 8-12 weeks old) were obtained from the Experimental Animal Center of Jilin University. All animal studies were performed according to the ethical guidelines of the care of animal life and approved by the ethics committee of Jilin University.

Biodistribution Twelve healthy female KM mice were used for the study of the biodistribution of cisplatin and curcumin released from cis-cur/fiber. Mice were randomly divided into two equal groups: (1) subcutaneous implantation of cis-cur/fiber (1 cm^2 in size, 0.15 mg of cisplatin and 0.5mg curcumin in content) at the right armpit of mice; (2) 0.15 mg of cisplatin injected intravenously (i.v) and 0.5 mg curcumin injected intraperitoneally(i.p), which is coded as cis-cur/i.v-i.p. The animals were sacrificed at 1h, 10 h and 24h after drug administration and samples of liver, kidneys, and underarm muscle tissues were harvested. Then all samples were divided into two parts, one of which were analyzed by ICP-MS for cisplatin, and the other of which were assessed by high performance liquid chromatography (HPLC) for curcumin. The chromatographic conditions were as follows: Agilent Technologies 1200 Series (150 mm x 4.6mm, 5 μm) was used; the mobile phase was the mixture of methanol and water (including 1% citric acid) at a volume ratio of 70:30 and the flow rate was 0.8 ml · min−1; the wavelength of the UV detector was 420 nm.

Prevention of tumor recurrence *in vivo* A total of 2×10^{6}ml^{-1} U14 cells in PBS (0.1 ml) were subcutaneously injected into the right armpit of mice. After tumors reaching a volume of 100 - 200 mm^3, tumors were surgically removed by sharp dissection under anesthesia through a neighboring incision to the tumor. After all visible tumor tissue was removed, postoperative mice were randomly divided into three groups (8 mice in group1, 7 mice in group2 or group3): (1) implantation of cis-cur/fiber (1 cm^2 in size, 0.15 mg cisplatin and 0.5 mg curcumin in content) at the site of tumor resection. (2) 0.15 mg of cisplatin (i.v) and 0.5 mg curcumin(i.p) (3) only tumor resection.

Mice were monitored every 3 days for the evidence of local tumor recurrence, the tumor volume and body weight was measured simultaneously. Time of local recurrence was defined as the day of detection of a palpable subcutaneous nodule (approximately 2 × 2 ×1 mm^3) in the armpit of mice. At 14 days, one animal from each group was taken a picture to show the visible morphology.

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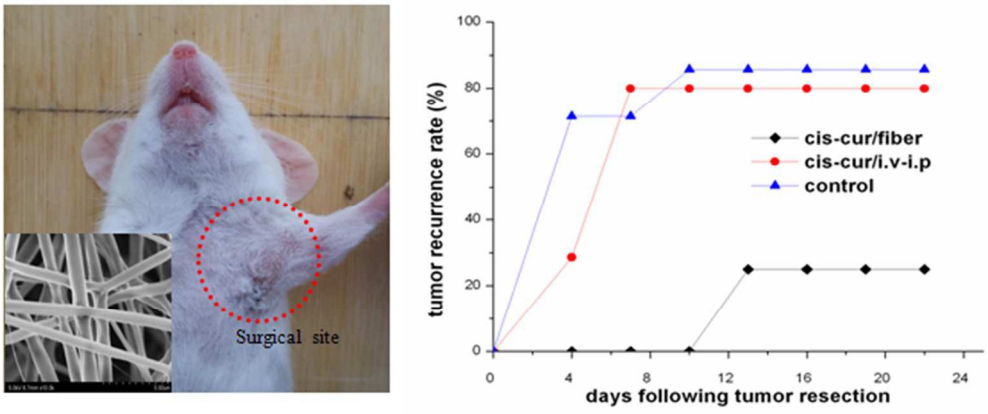

Electrospun nanofibers co-loaded with cisplatin and curcumin effectively reduce the risk of local cervical cancer recurrence after surgery

250x128mm (300 x 300 DPI)