Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 1047–1056

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Use of asymmetric multilayer polylactide nanofiber mats in controlled release of drugs and prevention of liver cancer recurrence after surgery in mice

Shi Liu, MSc[a,c], Xue Wang, PhD[d], Zhiyun Zhang, MSc[a,c], Yan Zhang, PhD[e], Guangyuan Zhou, PhD[b,*], Yubin Huang, PhD[a], Zhigang Xie, PhD[a,*], Xiabin Jing, BS[a]

[a]*State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, People's Republic of China*

[b]*Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, People's Republic of China*

[c]*The University of Chinese Academy of Sciences, Beijing, People's Republic of China*

[d]*Health Improving Department, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China*

[e]*The First Hospital of Jilin University, Changchun, People's Republic of China*

Received 8 September 2014; accepted 1 March 2015

Abstract

Local tumor recurrence remains a major clinical problem following surgical treatment for most cancers such as hepatocellular carcinoma (HCC). An implantable local drug delivery system may be suitable for addressing this unmet clinical need. In this study, asymmetric multilayer polylactide nanofiber (AMPN) mats were prepared and a one-sided and prolonged release profile of hydrophilic dye or oxaliplatin was observed after they were sandwiched between two liver lobes in mice. Covering the surgery site by drug-loaded AMPN mat after tumor resection, in both subcutaneous and orthotopic HCC model in mice, the recurrence of HCC was significantly retarded and the survival time of mice was markedly prolonged. In conclusion, post-surgical therapy at tumor resection margins by drug-loaded AMPN mats may represent a suitable application of nanofiber-based local chemotherapy.

Key words: Cancer recurrence; Drug-loaded nanofiber mats; HCC treatment; Local therapy; One-sided drug release; Orthotopic liver model

Recurrence of hepatocellular carcinoma (HCC) occurs in 50%-80% of the HCC patients within 2-5 years after resection. The typical mechanism is de novo tumor formation in the cirrhotic liver, or intrahepatic metastases that were too small to be detected/identified at the time of resection. The risk of local recurrence is correlated with the distance between the surgical margin and the primary tumor.[1-3] Therefore, to kill the remaining cancer cells after resection is the right choice to prevent HCC recurrence.

Recently, site-specific and controlled delivery of antitumor drugs by using biodegradable polymer electrospun nanofibers as drug carriers seems to be a promising method owing to their specific properties such as nanoscale size and high specific surface area, high enough drug content and controlled release of the loaded drugs, and ease in manufacturing and handling.[4-7]

In a previous study we found that doxorubicin-loaded poly(L-lactide) (PLA) electrospun nanofibers which were applied on the cancer surface could effectively inhibit the growth of liver cancer in mice and caused very low systemic toxicity, indicating that nanofiber-based local drug delivery system is potential as a new, reasonable, easy-to-use, and low-cost therapeutic option for HCC patients.[5] However, some major shortcomings were found, including rapid release of doxorubicin·HCl from the nanofibers (about 90% drug was

Abbreviations: AMPN, asymmetric multilayer polylactide nanofiber; CPA, cyclophosphamide monohydrate; Flu, fluorescein sodium salt; HCC, hepatocellular carcinoma; HPLC, high performance liquid chromatography; ICP-MS, Inductively coupled plasma mass spectrometry; IHC, Immunohistochemistry; KM, Kunming mice; M5 M3, M2, multi-layer composite electrospun fiber mats as shown in Figure 1; NIR17, a brand of near infrared cyanine fluorescent dye; Oxa, oxaliplatin; OxCy, a mixture of oxaliplatin and cyclophosphamide; PBS, phosphate buffered saline; PLA, poly(L-lactide).

Declaration of Interests: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This work is funded by the National Natural Science Foundation of China (Project No. 21004062, 51103148, and 51373167), and by the Ministry of Science and Technology of China ("973 Project", No. 2009CB930102).

*Corresponding authors.

E-mail addresses: gyzhou@ciac.ac.cn (G. Zhou), xiez@ciac.ac.cn (Z. Xie).

http://dx.doi.org/10.1016/j.nano.2015.03.001
1549-9634/

released within 6 h when adhered on the surface of the liver), drug release into the abdominal cavity as well, and high risk of tissue adhesion between the nanofibers-covered liver and peritoneum.

In this study, therefore, a drug-loaded asymmetric multilayer PLA nanofiber (AMPN) mats were designed to overcome the drawbacks mentioned above. As shown in Figure 1, *A* (M5), the mat is composed of five layers: (i) the top layer of PLA film, separating the tumor from abdominal cavity or from the other liver lobes, which serves as a physical barrier to produce a one-sided release pathway and to prevent tissue adhesion, (ii) first nanofiber layer loaded with anticancer drug or fluorescent dye, (iii) blank nanofiber layer as second barrier to delay the release of drug from (ii) toward the tumor, (iv) second drug- or dye-loaded nanofiber layer similar to (ii), (v) the bottom layer of blank nanofiber layer, which is adhered on the tumor and serves as the last barrier.

To prepare the AMPN mats, PLA was selected to make the non-porous film (i) and the porous electrospun fiber layers (ii) to (v) because of its biocompatibility and biodegradation. Hydrophilic and hydrophobic fluorescent dyes were simultaneously loaded into the layers (ii) and (iv) as model drugs to evaluate the one-sided and prolonged release. Oxaliplatin (abbr. as Oxa) alone or a mixture of oxaliplatin and cyclophosphamide (abbr. as OxCy) was electospun into the layers (ii) and (iv) as real therapeutic agent to examine the anti-tumor effect of the mats.

To evaluate the *in vivo* efficacy of the mats against HCC, the drug-loaded AMPN mats were placed at surgery site after tumor resection in both subcutaneous and orthotopic HCC models in mice. The purpose of the present study is to seek the possibility and feasibility of the application of the drug-loaded AMPN mats in the prevention of HCC recurrence after surgery (Figure 2).

Methods

Materials

Preparation and characterization of fluorescent dye- or anticancer drug-loaded AMPN mats are described in the Supplementary Material (SM). The AMPN mats prepared are abbreviated according to their composition and layer numbers. “M5”, “M3” and “M2” designate total layers of the mats, as shown in Figure 1, *A*. “Oxa”, “OxCy”, “Dye”, and “PLA” stand for the additives spun into the layer (ii) and (iv), with “Oxa” for oxaliplatin (10% with respect to PLA used), “OxCy” for oxaliplatin plus cyclophosphamide monohydrate (7.5% each), “Dye” for fluorescein sodium salt (Flu) plus near infrared cyanine fluorescent dye NIR17 (10% each), and “PLA” for no additives. For example, Oxa/M5 means an asymmetric five-layer mat in Figure 1, *A* with layer (ii) and (iv) containing 10% oxaliplatin, Dye/M3 means an asymmetric three-layer mat in Figure 1, *A* with layer (ii) and (iv) containing 10% Flu and 10% NIR17, and PLA/M2 means a blank PLA mat of two layers without any additives.

Animals and cell lines

Male Kunming mice with body weight ranging from 25 to 40 g were provided by the Experimental Animal Center of Jilin University. The study protocol was approved by the local institution review board and performed according to the Guidelines of the Committee on Animal Use and Care of Chinese Academy of Sciences. Murine hepatoma H22 cell line was a gift of Jilin University.

In vitro release from Dye- and OxCy-containing fiber-mats

The accurately weighed samples of Dye/M5, Dye/M3 and Dye/M2 were incubated at 37 °C in 20 ml of phosphate buffered saline (PBS, pH 7.4) in the presence of proteinase K (≥30 U/mg protein, 5 μg/ml) in a thermostated shaker. At predetermined time intervals, 1.0 ml of the released solution was withdrawn for ultraviolet (UV) analysis to detect Flu and NIR17 concentration and equal amount of fresh buffer solution was added back.

The protocol of OxCy/M5, OxCy/M3, OxCy/M2 was similar. Inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC) were used to detect Oxa and CPA, respectively. The chromatographic conditions were as follows: a Dikma Technologies C18 reversed-phase column (250 mm × 4.6 mm, 5 μm) was used; the mobile phase was the mixture of water and acetonitrile at a volume ratio of 65:35 and the flow rate was 1.0 $ml \cdot min^{-1}$; and the wavelength of the UV detector was 195 nm.

Assessments of one-sided and prolonged release of Dye/M5 and Oxa/M5 in vivo

Twenty-one healthy male KM mice were used to study the dye release and diffusion from Dye/M5 *in vivo*. The mice were randomly divided into three groups for the placement of Dye/M2, Dye/M3 and Dye/M5. After surgical laparotomy and exposure of the liver, the test mats (25 mm^2) were directly sandwiched between ventral aspect of the middle liver lobe (coded as M-liver) and dorsal aspect of left liver lobe (coded as L-liver). The bottom layer (v) (Figure 1, *A*) was toward M-liver and the top layer (i) was toward L-liver. The animals were sacrificed at 1 h, 6 h, and 1, 3, 7, 14 days after mat implantation and samples of liver, kidneys, spleen, lungs, and heart were harvested. After removal of the residual mats from the liver, the excised organs were imaged by fluorescent imaging system (CRI Maestro 500FL, MA, USA) to follow the release of dyes from the mats and their biodistribution in mice. Fixed exposure time was adopted for all specimens.

To further verify the results of fluorescence imaging, the release behavior of Oxa/M5 was studied *in vivo*. The implantation protocol of Oxa/M2, Oxa/M3 and Oxa/M5 was similar to that of Dye/M5. All samples including the residual mats, kidneys, M-liver and L-liver were dissolved in the concentrated nitric acid, diluted by the distilled water, and analyzed by ICP-MS to detect the contents of Oxa in residual material (1, 3, 6, 12, and 24 h) and mice organs (1, 12, and 24 h). Then the cumulative release rate of the Oxa *in vivo* was calculated. One thing to note here is that L-liver in this case includes all remnant liver except middle liver lobe (M-liver) rather than the dorsal aspect of left liver lobe alone mentioned above in the case of fluorescent imaging.