Electrospun 5-fluorouracil loaded bovine serum albumin-polyvinylpyrrolidone nanofibers

Upulitha E. Illangakoon, Nicholas P. Chatterton, Gareth R. Williams, and Tahir Nazir

School of Human Sciences, Faculty of Life Sciences, London Metropolitan University, 166-220 Holloway Road, London N78DB, UK, e.illangakoon@yahoo.com

ABSTRACT

5-FU loaded bovine serum albumin (BSA)/ polyvinylpyrrolidone (PVP) nanofibers prepared using an electrospinning process. The nanofibers were characterised by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). Nuclear magnetic resonance was used to confirm that the drug was not substantially degraded by the electrospinning process.

SEM images showed that the fibers formed were cylindrical in shape with smooth surfaces. FTIR indicated that BSA and 5-FU are well distributed in the fiber, forming significant numbers of H-bonds with the carrier PVP polymer. The XRD results demonstrated that BSA and 5-FU were distributed in the fiber matrix in an amorphous state. *In-vitro* dissolution tests showed that the fiber mats released the drug within 10 s through a polymer-controlled mechanism, leading to a significant decrease in dissolution time over the pure drug. This enhanced dissolution property suggests that the 5-FU loaded BSA/PVP fibers have potential in fast-dissolving drug delivery systems.

Keywords: Electrospinning, 5-Fluorouracil, Bovine Serum Albumin, Polyvinylpyrrolidone

1 INTRODUCTION

Nanoscale drug delivery systems are becoming increasingly prevalent in pharmaceutical applications. Over the past few years, cancer treatments have been greatly improved by new tools based on nanotechnology. However, the targeted delivery of anti-cancer drugs to tumor tissue remains one of the most challenging goals in the pharmaceutical field due to the pharmacologically specific environment in the tumor [1]. One solution to this challenge is to use albumin, which accumulates in solid tumors and hence is an attractive agent for tumor targeting drug delivery systems [2 – 5].

Electrospun nanofibers have attracted much attention in the field of drug delivery because of their high surface-area-to-volume ratio, flexibility, and controllable morphology [6-10]. They have the potential to ameliorate the systemic side effects of anticancer drug therapy and to improve selective toxicities against cancer cells. In electrospinning, a solution of a carrier polymer and a functional component is ejected from a syringe towards a metal "collector" plate. An electrical voltage sufficient to overcome the surface tension of the solution is applied between the syringe and collector, which leads to the production of non-woven mats of very fine fibers [6].

The drug 5-Fluorouracil (5-FU) has a broad spectrum of activity against solid tumors but it suffers from a short half life due to rapid metabolism, and also exhibits non selective activity against healthy cells [5,11]. In this study, we aimed to combine albumin and 5-FU into nanofibers for precisely targeted anti-tumour medications. This builds on previous work by other authors studying the interaction between 5-FU and BSA [12-13] and developing 5-FU loaded chitosan scaffolds [14]. Albumin based microspheres [15] and nanoparticles [3] have also been loaded with anti-cancer drugs, and found to have potent activity.

The aim of the study is to produce the first electrospun albumin / anti-cancer drug nanofibers. 5-FU loaded nanofibers were prepared with polyvinylpyrrolidone (PVP) as a biocompatible polymer. The fibers also incorporated BSA to enhance selectivity. The fiber morphology, interaction between the drug and polymer, dissolution properties, and the stability of the composite fibers have additionally been sudied, and their *in vitro* efficacy probed.

2 MATERIALS AND METHODS

2.1 Materials

5-FU, bovine serum albumin, PVP 360, formic acid and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide were purchased from Sigma Aldrich and used as received. Cell culture reagents were procured from PAA Laboratories.

2.2 Methods

Electrospinning

A series of 5-FU loaded BSA/PVP fibers were prepared using 10% PVP 360 (*Mw = 360 kDa)* with differing amounts of BSA and 5FU in a solvent system comprising 80 % ethanol and 20% formic acid. During the electrospinning process, the feed rate was fixed at 1.25 mL/h, and a square (17 x 17 cm^2) of aluminium foil was used as a grounded target. A high-voltage DC power supply (Fug Elektronik) was used to maintain a voltage of 20 kV between the spinneret (3 mm internal diameter) and collector, with a spinneret to collecting distance of 12 cm. All electrospinning processes were carried out under ambient conditions (temperature 21±1 ºC, relative humidity 35 ±3 %). The electrospun fibers were dried and stored in a vacuum dessicator until further use.

2.3 Characterization of fibers

2.3.1 Morphology

SEM images were recorded on a (SEM, FEI Quanta 3D) scanning electron microscope equipped with an energy-dispersive X-ray spectroscopy (EDS) accessory.

2.3.2 Physical state of the components in the drug-loaded fibers

Differential scanning calorimetry (DSC) analyses were carried out using an MDSC 2910 calorimeter (TA Instruments Co., USA). Sealed samples were heated at 10 °C/ min from 40 to 350 °C in air. X-ray diffraction (XRD) patterns were obtained on a Philips PW1830 instrument using Cu Kα radiation at 40mV and 25 mA.

2.3.3 Compatibility between the components of drug-loaded fibers

Fourier transform infrared spectroscopy (FTIR) was conducted using a Bruker Vector 22 FTIR spectrometer fitted with an ATR attachment. The scanning range was 500 – 4000 cm^{-1} and the resolution 1 cm^{-1}.

2.3.4. Fast dissolution properties

8 mg of the fiber mat was placed in petri dish containing 10 mL of phosphate buffer (pH 7.4) at room temperature. and the time for the fiber to disappear recorded. Experiments were carried out in triplicate.

2.4 *In vitro* cytotoxicity assay

Cells were seeded on a 96 well plate with a density of 10,000 cells/mL. The anti-tumor activity of the fibers was initially assessed using the THP-1 cell line and subsequently by human epidermal keratinocyte (HEK) cells. The morphology of the cells was studied with a light microscope and cell viability was calculated using the MTT assay.

3. Results and conclusion

Three BSA/5-FU/PVP fibers were prepared, along with controls, as detailed in Table 1.

Table 1: Details of the fibers prepared

	F0	F1	F2	F3	F4	F5
% BSA	5	5	5	8	0	PM
% 5-FU	0	4	7	13	7	

PM denotes a physical mixture of 7 % FU, 5 % BSA and 88% PVP.

Scanning electron microscopy (SEM) images (Figure 1) showed that the fibers formed were cylindrical in shape with smooth surfaces, and average fiber diameters of around 400 nm.

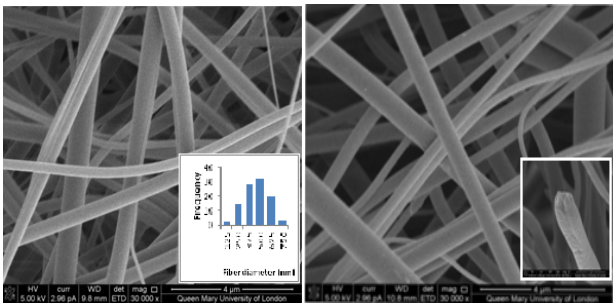

Figure 1. SEM images of a) F3 b) F2

EDS spectra obtained from different regions of the fiber mat showed a consitant F content, suggesting that 5-FU is uniformly distributed in the fiber mat.

FTIR spectra (shown in Figure 2) indicated that the BSA and 5FU are well distributed in the fiber, forming significant numbers of H-bonds with the carrier PVP polymer. In particular, a comparison with the physical mixture suggested that more H-bonds were formed in the composite fibers, which suggests there may be enhanced thermodynamic stability relative to the physical mixture.

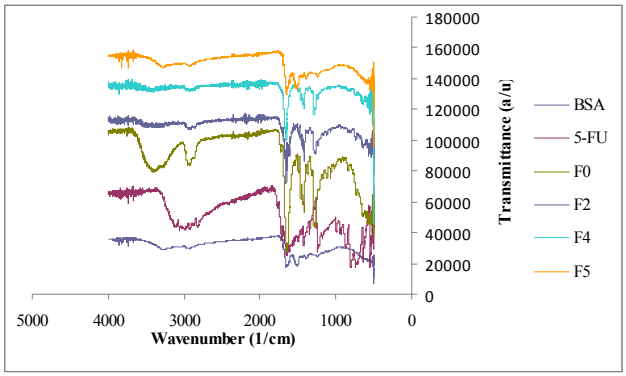

Figure 2. IR spectra of selected composite fibers, 5-FU, BSA, PVP/5-FU fibers (F4) and a physical mixture (denoted F5)

The XRD patterns of pure 5-FU (Figure 3(a)) shows characteristic diffraction peaks of the drug, showing it to be present as crystalline material. Patterns of the composite fibers, however, show broad humps typical of amorphous forms. There are no peaks corresponding to crystalline 5-FU detectable in the XRD patterns of the composite fibers (Figure 3(b)) indicating that the 5-FU in the fibers was no longer present as crystalline material, but had been converted into an amorphous state. This may either be as amorphous molecular aggregates or as a solid solution in the fiber. The XRD pattern of a physical mixture of 5-FU and PVP (not shown) contained all the characteristic reflections of 5-FU, demonstrating that electrospinning is needed to render the drug into an amorphous state.

DSC results (not shown) concurred with the XRD results to show that BSA and 5-FU were distributed in the fiber matrix in an amorphous form,.

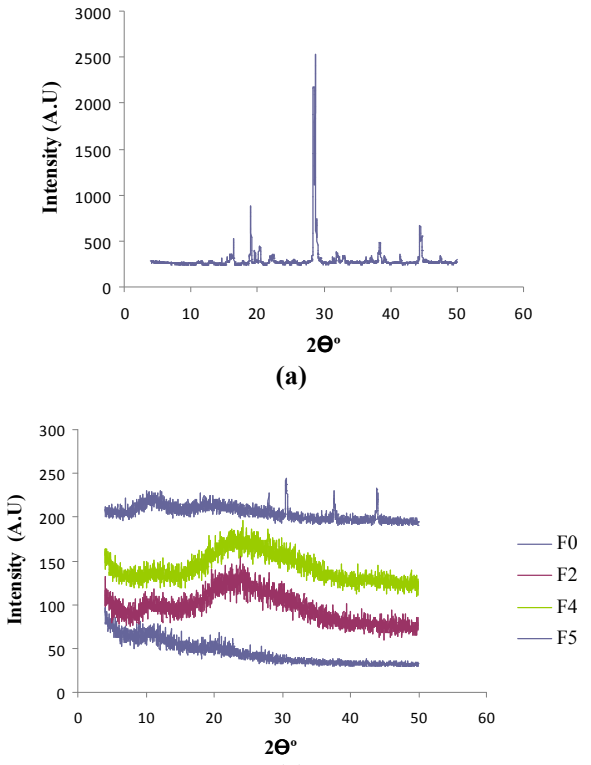

Figure 3. XRD patterns of (a) 5-FU and (b) selected 5-FU containing nanofibers.

In-vitro dissolution tests showed that the fiber mats dissolved completely, releasing the drug within 10 s, a significant decrease in dissolution time over the pure drug: this suggests that the 5-FU loaded BSA/PVP fibers may have potential in fast-dissolving drug delivery systems.

Preliminary experiments indicate that the 5-FU loaded fibers inhibit the proliferation of THP-1 cells and HEK cells. Further experiments are ongoing to accurately quantify this effect.

In conclusion, for the first time we have prepared albumin-based nanofibers for targeted anti-cancer drug delivery; preliminary data indicate that these fibers improve the solubility of 5-FU and are effective against model cancel cell lines. We believe that the fibers may have potential applications as fast-dissolving drug delivery systems, and as patches for dermal treatments.

REFERENCES

[1].Qiao, W. et al., 2010. Cancer Therapy Based on Nanomaterials and Nanocarrier Systems. *Journal of Nanomaterials*, 2010.

[2].Patil, G.V., 2003. Biopolymer albumin for diagnosis and in drug delivery. *Drug Development Research*, 58(3), pp.219-247.

[3].Karmali, P.P. et al., 2009. Targeting of albumin-embedded paclitaxel nanoparticles to tumors. *Nanomedicine□: nanotechnology, biology, and medicine*, 5(1), pp.73-82.

[4].Kratz, F., 2008. Albumin as a drug carrier□: Design of prodrugs , drug conjugates and nanoparticles. *Journal of Controlled Release*, 132(3), pp.171-183.

[5].Maghsoudi, A., Shojaosadati, S.A. & Farahani, E.V., 2008. 5-Fluorouracil-Loaded BSA Nanoparticles□: Formulation Optimization and In Vitro Release Study. *Optimization*, 9(4), pp.1092-1096.

[6].Huang, Z.-ming et al., 2003. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Most*, 63, pp.2223-2253.

[7].Ma, G. et al., 2011. Paclitaxel loaded electrospun porous nanofibers as mat potential application for chemotherapy against prostate cancer. *Carbohydrate Polymers*, 86(2), pp.505-512.

[8].Xu, X. et al., 2008. The release behavior of doxorubicin hydrochloride from medicated fibers prepared by emulsion-electrospinning. *European Journal of Pharmaceutics and Biopharmaceutics*, 70, pp.165-170.

[9].Chen, P. et al., 2010. European Journal of Pharmaceutics and Biopharmaceutics A controlled release system of titanocene dichloride by electrospun fiber and its antitumor activity in vitro. *European Journal of Pharmaceutics and Biopharmaceutics*, 76(3), pp.413-420.

[10].Xu, Xiuling et al., 2006. BCNU-loaded PEG – PLLA ultrafine fibers and their in vitro antitumor activity against Glioma C6 cells. *Journal of Controlled Release*, 114, pp.307-316.

[11].Rejinold, N.S. et al., 2011. International Journal of Biological Macromolecules 5-Fluorouracil loaded fibrinogen nanoparticles for cancer drug delivery applications. *International Journal of Biological Macromolecules*, 48(1), pp.98-105.

[12].Hu, Y.-jun et al., 2005. Studies on the interaction between 1-hexylcarbamoyl-5-fluorouracil and bovine serum albumin. *Journal of Molecular Structure*, 738, pp.143-147.

[13]. Bojko, B. & Sułkowski, W., 2003. Interaction of anticancer drugs with human and bovine serum albumin. *Journal of Molecular Structure*, 653, pp.133-140.

[14].Gao, H., Gu, Y. & Ping, Q., 2007. The implantable 5-fluorouracil-loaded poly (L -lactic acid) fibers prepared by wet-spinning from suspension. *Journal of Controlled Release*, 118, pp.325-332.

[15].Lu, X.-yun, Zhang, Y. & Wang, L., 2010. Preparation and In Vitro Drug-Release Behavior of 5-Fluorouracil-Loaded Poly (hydroxybutyrate- co-hydroxyhexanoate) Nanoparticles and Microparticles. *Polymer*.

[16].Shen, X. et al., 2011. Electrospun diclofenac sodium loaded Eudragit ® L 100-55 nanofibers for colon-targeted drug delivery. *International Journal of Pharmaceutics*, 408(1-2), pp.200-207.

Acknowledgments

We thank London Metropolitan University for provision of a Vice Chancellor's PhD Scholarship to UEI, and the EPSRC for funding access to the SEM at Nanoforce, Queen Mary, University of London. We also thank Ursula for her help with the SEM.