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Electrospun gelatin/sodium bicarbonate and poly(lactide-*co*-ε-caprolactone)/sodium bicarbonate nanofibers as drug delivery systems

Qingqing Sang[a], Gareth R. Williams[b], Huanling Wu[a], Kailin Liu[a], Heyu Li[a], Li-Min Zhu[a,c,*]

[a] College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China
[b] UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London WC1N 1AX, UK
[c] Key Laboratory of Science & Technology of Eco-Textiles, Ministry of Education, Donghua University, Shanghai 201620, China

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ABSTRACT

In this work, we report electrospun nanofibers made of model hydrophobic (poly(lactide-*co*-ε-caprolactone); PLCL) and hydrophilic (gelatin) polymers. We explored the effect on drug release of the incorporation of sodium bicarbonate (SB) into these fibers, using the potent antibacterial agent ciprofloxacin as a model drug. The fibers prepared are smooth and have relatively uniform diameters lying between *ca.* 600 and 850 nm. The presence of ciprofloxacin in the fibers was confirmed using IR spectroscopy. X-ray diffraction showed the drug to be incorporated into the fibers in the amorphous form. *In vitro* drug release studies revealed that, as expected, more rapid drug release was seen with gelatin fibers than those made of PLCL, and a greater final release percentage was obtained. The inclusion of SB in the gelatin fibers imparts them with pH sensitivity: gelatin/SB fibers showed faster release at pH 5 than pH 7.4, while fibers without SB gave the same release profiles at both pHs. The PLCL fibers have no pH sensitivity, even when SB was included, as a result of their hydrophobic structure precluding the ingress of solvent. *In vitro* cell culture studies showed that all the fibers are able to promote cell proliferation. The ciprofloxacin loaded fibers are effective in inhibiting *Escherichia coli* and *Staphylococcus aureus* growth in antibacterial tests. Thus, the gelatin-based fibers can be used as pH-responsive drug delivery systems, with potential applications for instance in the treatment of tumor resection sites. Should these become infected, the pH would drop, resulting in ciprofloxacin being released and the infection halted.

1. Introduction

Nanoscale fibers have been increasingly explored in recent years. There are many approaches available for preparing these, such as template polymerization, self-assembly, phase separation, and electrostatic spinning. The latter has attracted particular attention for the preparation of nanofiber materials because of its simplicity, low cost, and the wide variety of materials which can be processed [1,2]. Nano- and micro-scale fibers from electrospinning find use in a range of areas including catalysis, environmental remediation, and for the delivery of active pharmaceutical ingredients [3]. Electrospinning has attracted particular attention for biomedical applications, mainly due to growing interest in nano-technologies and the attractive material properties which can be obtained (*e.g.* porosity, flexibility) [4]. For instance, researchers have explored the use of drug-loaded electrospun fibers for the treatment or prevention of cancer. Ranganath et al. [5] prepared electrospun poly(lactic-*co*-glycolic acid) nanofibers loaded with the anti-cancer drug paclitaxel, and found them to be effective against glioma tumors *in vivo*. In other work, Liu et al. [6] prepared electrospun fibers containing sodium dichloroacetate and found these to lead to the necrosis of tumor cells, resulting in an inhibition rate of up to 96%. Tissue engineering, such as for the nerve, has also been widely explored [7].

An ideal drug delivery system (DDS) must be both safe and effective, and should also offer a specific delivery pattern. This might comprise site-specific drug delivery or extended release, for instance. Electrospun fibers have great promise in this regard. To achieve the desired release properties, the choice of polymer to be used requires careful consideration. A fast-dissolving polymer will give more rapid release than an insoluble material, but the solubility of the drug in the polymer is also important. In a study by Natu and co-workers [8], acetazolamide and timolol maleate-loaded fibers of poly(ε-caprolactone) and poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) were prepared by blend electrospinning. Maleate was released faster than acetazolamide. High drug loadings, where crystals of the active ingredients were present, showed a greater burst release and faster release rate than low drug content fibers where the drug was spread evenly throughout the (slow-dissolving) polymer. Both the fiber composition and drug solubility in

* Corresponding author at: College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China.
E-mail address: lzhu@dhu.edu.cn (L.-M. Zhu).

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the polymer clearly influenced the release profiles seen.

Fibers can also be electrospun from stimuli-responsive materials, for instance those that change their properties with a change in temperature [9] or pH. The latter is particularly important given the changes in pH which occur as a formulation taken orally passes through the human gastrointestinal tract, and differences between the physiological pH and that of the tumor microenvironment. A number of pH-sensitive drug delivery systems generated by electrospinning have been reported. For example, Aguilar et al. [10] prepared composite fibers of polyurethane and the pH-sensitive polymer Eudragit® L100-55 loaded with paclitaxel. *In vitro* drug release experiments showed the composite mats give slow release of paclitaxel at pH 4, and relatively rapid release at pH 6. Adopting a different approach, Zhao and co-workers [11] developed an acid-responsive nanofiber scaffold using electrospinning. The fibers comprised poly(L-lactic acid) loaded with sodium bicarbonate and ketoprofen. In acidic conditions, sodium bicarbonate reacts with protons to form CO_2 gas. This reaction generates pores in the fibers, and thus permits a drug cargo to diffuse easily into solution. In contrast, at neutral pH the SB is stable, and thus the fiber remains non-porous and impermeable. In this way, pH sensitivity can be achieved without the need for bespoke polymers, allowing a much wider range of responsive systems to be developed.

Our ability to treat and cure cancer has improved tremendously in recent years [12]. However, there are still numerous deaths from cancer every year, and thus the search for new and more effective therapies continues. Surgical removal of the tumor is perhaps the “gold standard” treatment, where the growth is accessible. This comes with potential problems though: for instance, the operation may cause an inflammatory reaction, which in turn can lead to the recurrence of the tumor [13]. This reaction is also associated with a lowering of the local tissue pH, and thus a pH-responsive system might be beneficial here [14].

In this study, we report electrospun fibers developed using sodium bicarbonate (SB) as a pH-responsive component. We explored the ability of SB to impart pH sensitivity on two polymers which do not normally possess this: gelatin and poly(lactide-*co*-ε-caprolactone). These are respectively model hydrophilic and hydrophobic polymers, and this is the first time that the ability of sodium bicarbonate to induce pH-sensitivity in analogous nanofibers of differing hydrophilicity has been investigated. The ultimate aim of this work is to develop novel anti-tumor therapeutics.

Gelatin is a hydrophilic naturally-occurring biopolymer. It has been widely used in food, medicine and cosmetic applications as well as in biomaterials [15,16], and is the major structural protein in animal skin and bones. Gelatin has very good biological compatibility, and thus is attractive as a potential drug carrier [17]. However, gelatin dissolves quickly into aqueous media (regardless of pH), and thus although gelatin fibers can be prepared by electrospinning they undergo very rapid release unless some post-fabrication modification is performed. Approaches to ameliorate this issue include using a layer-by-layer approach to combine multiple polymers in a single system. For instance, Mandal et al. [18] prepared a silk fibroin/gelatin multilayer film and were able to control the release of the drug incorporated. Such a fabrication process is relatively complex though, and a simple option would be advantageous. Here, we opted to simply cross-link the gelatin molecules in the fibers after their fabrication, using glutaraldehyde as a crosslinking agent [19]. Zhang and co-workers have previously demonstrated the utility of this approach by employing a saturated glutaraldehyde vapor to improve the thermostability and mechanical properties of gelatin nanofibers [20].

Poly(lactide-*co*-ε-caprolactone) (PLCL) is a hydrophobic aliphatic polyester copolymer. It has the advantages of good biocompatibility, a lack of toxicity *in vivo*, and high mechanical strength. It is also easy to process by electrospinning, and PLCL products have been explored in tissue engineering, as well as for wound dressings, drug delivery, and other applications [21,22].

The model drug ciprofloxacin was loaded into both the PLCL and gelatin systems being explored. Ciprofloxacin is a third generation quinolone, with broad-spectrum antibacterial activity [23,24]. By developing novel pH-responsive systems containing ciprofloxacin, we hoped to develop scaffolds which ultimately might be used after tumor resection surgery. A detailed comparison of the drug release from hydrophobic PLCL and hydrophilic gelatin-based fibers is reported, and their potential applications studied.

2. Experimental

2.1. Materials

Gelatin (powder, purity > 99.5%) was supplied by the Sinopharm Chemical Reagent Co., Ltd.), while dimethyl sulfoxide (DMSO) was sourced from J & K Chemicals. Dulbecco's Modified Eagle Medium (DMEM) was provided from Jinuo Biological Medicine Technology Ltd. Poly(lactide-*co*-ε-caprolactone) (PLCL, PCL:PLLA = 1:1) came from Jinan Daigang Biomaterials. 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP, purity > 95.5%), ciprofloxacin (purity > 98%), phosphate-buffered saline (PBS, [sodium phosphate] = 10 mM, [NaCl] = 150 mM; pH = 7.4), sodium bicarbonate (SB, powder, purity > 99.5%), penicillin/streptomycin, and thiazolyl blue tetrazolium bromide (MTT) were supplied by Sigma-Aldrich Ltd. L929 cells were purchased from the Chinese Institute of Biochemistry and Cell Biology. An acetic acid buffer (AA, [sodium acetate] = 25 mM; pH = 5.0) was prepared in-house. All other chemicals were at least analytical grade, and water was doubly distilled prior to use.

2.2. Electrospinning

Gelatin or PLCL were dissolved in hexafluoroisopropanol (HFIP) with or without SB, as detailed in Table 1. Ciprofloxacin was added into selected solutions at a drug to polymer ratio of 1:9 (w/w). The solutions were magnetically stirred at room temperature for 24 h in closed glass bottles sealed with parafilm. They were then loaded into 5 mL plastic syringes fitted with a stainless steel spinneret (internal diameter 0.5 mm). A syringe pump (KDS100, Cole-Parmer) was used to drive the working fluids, at a flow rate of 0.8 mL/h. The electrospinning process was carried out with a high voltage power supply (ZGF-2000, Shanghai Sute Electrical Co. Ltd.) used to provide a potential difference of 15 kV between the spinneret and collector plate. The latter had dimensions of 8 × 9 cm and was covered in aluminum foil. The distance between the spinneret and collector was 23 cm. Experiments were performed at 20–25 °C, and 33–45% relative humidity. The electrospun products were dried in a vacuum oven (DZF-6050, Shanghai Laboratory Instrument Work Co. Ltd.) for two days at 25 °C. After drying, the gelatin fibers were crosslinked using an aqueous glutaraldehyde solution (25.0–28.0% (v/v)) to render them insoluble in water. The glutaraldehyde solution (10 mL) was placed at the bottom of a brown translucent desiccator, and the fiber samples loaded in a Petri dish and mounted on a rack above this solution for approximately 8 h. All further

Table 1
Details of the electrospinning solutions used in this study.

Fiber ID	Gelatin conc (w/v)	PLCL conc (w/v)	SB conc (w/v)	Ciprofloxacin conc (w/v)
G^+	15%	–	–	10%
G^-	15%	–	–	–
GS^+	15%	–	5%	10%
GS^-	15%	–	5%	–
P^+	–	5%	–	10%
P^-	–	5%	–	–
PS^+	–	5%	5%	10%
PS^-	–	5%	5%	–