European Journal of Pharmaceutical Sciences 103 (2017) 94–103

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Gefitinib/gefitinib microspheres loaded polyurethane constructs as drug-eluting stent coating

Weiluan Chen [a], Johanna Clauser [b], Anja Lena Thiebes [c], Donnacha J. McGrath [d], Nicola Kelly [d], Mies J. van Steenbergen [a], Stefan Jockenhoevel [c], Ulrich Steinseifer [b], Peter E. McHugh [d], Wim E. Hennink [a], Robbert J. Kok [a,*]

[a] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
[b] Department of Cardiovascular Engineering, Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Pauwelsstraße 20, 52074, Germany
[c] Department of Biohybrid & Medical Textiles (BioTex), AME-Helmholtz Institute for Biomedical Engineering, ITA-Institut für Textiltechnik, RWTH Aachen University, Aachen, Germany
[d] Biomechanics Research Centre, Biomedical Engineering, College of Engineering and Informatics, National University of Ireland, University Road, Galway, Ireland

ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form 28 January 2017
Accepted 2 February 2017
Available online 4 February 2017

Keywords:
Polyurethane
Polymeric microspheres
Drug-eluting stents
Bronchotracheal stent
Controlled release

ABSTRACT

One of the complications of bronchotracheal cancer is obstruction of the upper airways. Local tumor resection in combination with an airway stent can suppress intraluminal tumor (re)growth. We have investigated a novel drug-eluting stent coating for local release of the anticancer drug gefitinib.
A polyurethane (PU) sandwich construct was prepared by a spray coating method in which gefitinib was embedded between a PU support layer of 200 μm and a PU top layer of 50–200 μm. Gefitinib was either embedded in the construct as small crystals or as gefitinib-loaded poly(lactic-*co*-glycolic acid) (PLGA) microspheres (MSP). The drug was incorporated in the PU constructs with high recovery (83–93%), and the spray coating procedure did not affect the morphologies of the embedded microspheres as demonstrated by scanning electron microscopy (SEM), confocal laser scanning microscopy and fluorescence microscopy analysis. PU constructs loaded with gefitinib crystals released the drug for 7–21 days and showed diffusion based release kinetics. Importantly, directional release of the drug towards the top layer, which is supposed to face the tumor mass, was controlled by the thicknesses of the PU top layer. PU constructs loaded with gefitinib microspheres released the drug in a sustained manner for >6 months indicating that drug release from the microspheres became the rate limiting step. In conclusion, the sandwich structure of drug-loaded PLGA microspheres in PU coating is a promising coating for airway stents that release anticancer drugs locally for a prolonged time.

1. Introduction

Polyurethanes (PUs) are synthetic polymers that carry urethane (or carbothane) bonds (—NH—COO—) in their chains (Bayer, 1947), which represent an important category of biomedical polymers (Lamba et al., 1997). Over the past 40 years, PUs have been used as biomaterials (e.g. catheters and medical implants), as scaffolds for tissue engineering and as drug delivery systems due to their excellent mechanical flexibility, tailorable properties and good biocompatibility (Chen et al., 2013; Cherng et al., 2013; Zdrahala and Zdrahala, 1999).

Stents are hollow devices that are inserted in an obstructed natural passage (such as the coronary artery) to open and prevent its blockage (Chen et al., 2015; Lei et al., 2011). Since 1986, bare metal stents (BMS) have been used to treat stenosis (Puel et al., 1987; Roguin, 2011), however, one of the most common problems with these stents is neointimal proliferation (i.e., re-stenosis) (Chen et al., 2006). Therefore, drug-eluting stents (DES) that release antiproliferative compounds (such as paclitaxel, sirolimus and its derivatives, etc.) which can prevent neointimal hyperplasia have been developed (Hu et al., 2015; Seo et al., 2016; Sun et al., 2014).

Obstruction of the upper airways due to lung cancer can result in life threatening and distressing breathlessness (Herth and Eberhardt, 2016). Stent insertion can be a lifesaving procedure affording dramatic, emergent relief of acute respiratory distress from airway obstruction resulting extended survival (Hohenforst-Schmidt et al., 2016; Shaffer and Allen, 1998). PU membrane generally has a high tensile strength allowing them as a covering material for bronchotracheal stents that are compressed inside an introducer tube with a minimum volume during the delivery to the obstructed lumen (Kwon and Park, 2014; Lamba et al., 1997; Seo and Na, 2014). Anti-cancer drugs loaded in PU polymeric coating of stents are locally released to inhibit the growth of the

* Corresponding author at: Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands.
E-mail address: r.j.kok@uu.nl (R.J. Kok).

http://dx.doi.org/10.1016/j.ejps.2017.02.002

tumor while simultaneously minimizing systemic drug exposure (Puranik et al., 2013).

We now propose to load the anti-cancer drug gefitinib in the PU cover of a bronchotracheal stent. Gefitinib (Iressa®, ZD1839) is a low molecular weight drug (structure shown in Fig. 1) that competes with the binding of ATP to the intracellular tyrosine kinase domain of epidermal growth factor receptor (EGFR), thereby inhibiting receptor autophosphorylation and blocking downstream signal transduction (Barker et al., 2001; Wakeling et al., 2002). It has been approved for the treatment of EGFR-positive metastatic non-small cell lung cancer (NSCLC) (Reck et al., 2013).

Polymer-coated DES can either contain single or multiple layers (Lei et al., 2010; Simmons et al., 2008). Application of a single layer coating is most straight forward and technically easier to integrate in a product design, but multiple layers have the benefit to more precisely control drug release kinetics and direction. Lei et al. prepared a series of poly(ε-caprolactone) (PCL)-based multilayered films composed of a drug-free support layer and an antitumor drug (5-fluorouracil) containing top layer. The support layer of the construct blocks undesirable transport of the drug from the main drug layer towards the lumen of physiological tubular structure, thus achieving unidirectional drug release towards the tissue-contacting side of the stent (Lei et al., 2010).

In the present study, gefitinib particles were embedded in a nonwoven PU fleece that can be used for coating of nitinol stents. This type of bronchotracheal stent has recently been implanted in sheep (as an animal model that reflects the dimensions of the human upper airways) and was well tolerated for up to 6 months, with no signs of severe mucus plugging (Thiebes et al., 2016). These promising results encouraged us to develop drug-loaded stent coatings that can be applied in the same stent concept for palliative treatment of cancer-induced airway stenosis. PU constructs were prepared using a three-step method (as shown in Fig. 2) in which micronized drug crystals or drug loaded microparticles were applied between two non-woven PU layers, thus forming a sandwich-like structure. It has been reported that administration of medication via microspheres is advantageous because these systems can tailor the desired release profiles (Freiberg and Zhu, 2004). The thickness of the top layer was varied to get control over the release direction of the drug towards any remaining tumor masses in the bronchotracheal wall (see also Fig. 7B). We characterized the gefitinib loaded coatings by investigating a variety of properties like morphology, gefitinib release as well as the degradation behavior of the microspheres inside the PU constructs.

2. Materials and methods

2.1. Materials

PLGA 5004A (acid terminated, lactide/glycolide molar ratio 50:50, IV = 0.4 dl/g) was obtained from Corbion Purac Biomaterials, the Netherlands. Gefitinib (free base, >99%) was purchased from LC laboratories (Woodburn, MA, USA). Polyvinyl alcohol (PVA; M_W 30–70,000 g/mol; 87–90% hydrolyzed), disodium hydrogen phosphate (Na_2HPO_4), and sodium azide (NaN_3, BioUltra, ≥99.5%) were purchased from Sigma Aldrich (Germany). Chloroform was purchased from AppliChem, GmbH, Germany. Phosphate buffer saline (10 mM sodium phosphate, 140 mM NaCl, pH 7.4) was purchased from Braun (Melsungen AG, Germany), whereas dichloromethane (DCM) was purchased from Biosolve (Valkenwaard, the Netherlands). Medical grade carbothane PC-3575A (tradename Carbothane®) was obtained from Lubrizol, Belgium. Human fibrinogen (Mw = 341 kDa, lyophilized from 20 mM sodium citrate-HCl, pH 7.4, Calbiochem®) was purchased from Merck Millipore, and human thrombin and $CaCl_2$ were purchased from Sigma Aldrich, the Netherlands.

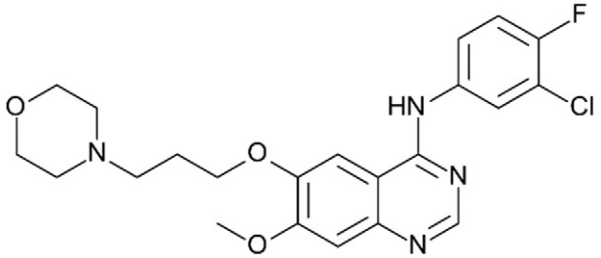

Fig. 1. Chemical structure of gefitinib.

2.2. Preparation of gefitinib-loaded microspheres

Drug-loaded PLGA microspheres were prepared by a solvent evaporation technique as described previously (Chen et al., 2016b). PLGA 5004A (5.0 g) was dissolved in 10 ml dichloromethane (DCM). A gefitinib stock solution with a concentration of 200 mg/ml was prepared by dissolving the drug in dimethyl sulfoxide (DMSO). Next, 2.5 ml of this gefitinib solution was added to the PLGA solution.

The obtained organic phase was added dropwise to a 2% PVA aqueous solution which was stirred at a rate of 30,000 rpm using an IKA homogenizer (IKA Labortechnik Staufen, Germany) for 6 min at room temperature. Next, the emulsion/suspension was stirred overnight at room temperature using a magnetic stirrer (500 rpm) to extract and evaporate DCM. Subsequently, the microspheres were collected by centrifugation (4000 g for 3 min, Laboratory centrifuge, 4K15 Germany), washed three times with 50 ml of distilled water, and lyophilized overnight. The obtained microspheres were suspended in 10 ml of water and subsequently put on stacked sieves (Nickel precision filters, Stork Veco BV, Eerbeek, The Netherlands) and then flushed with distilled water under reduced pressure. Gefitinib-loaded PLGA microspheres with sizes between 50 and 100 μm were obtained.

2.3. Preparation of PU coatings

2.3.1. Spray coating of the PU support layer

The PU support layer was fabricated using a spray coating method (Chen et al., 2016a; Nadzeyka et al., 2014) by a spraying device composed of a spraying unit of a nozzle (SATA Minijet 3000, SATA GmbH & Co. KG. Kornwestheim, Germany), a chloroform resistant polyamide hose (length = 120 cm), a syringe (50 ml, B. Braun Medical Inc., Germany) a syringe pump (LA-100, Landgraaf Labor systeme HLL GmbH, Germany), a rotating spindle (Φ = 22 mm, L = 20 cm) and a computer drive unit. The polymer solution was prepared by dissolving 7.5 wt% PC-3575A Carbothane in chloroform in the syringe, which was subsequently mounted on the syringe pump. The flow rate of the carbothane solution was set at 2 ml/min. The spraying duration was 3.5 min and the spraying air pressure was 0.8 bar. To achieve a uniform coating thickness, the spraying nozzle moved along the length of the spindle (rate = 31 mm/s). The spindle with the sprayed PU coating was then placed in an oven at 30 °C for 2 h to evaporate the chloroform.

2.3.2. Deposition of the gefitinib/gefitinib-loaded microspheres on the PU support layer

Drug/drug-loaded microspheres were applied onto the support layer by means of an in situ forming fibrin gel (Cederholm Williams et al., 2002), to affirm their tight adherence onto the Carbothane during the spraying process. Fibrin is commonly used in tissue engineering because of its biocompatibility (Breen et al., 2009; Rask et al., 2010).

Fibrinogen (90 mg) was dissolved in 3 ml Milli-Q water at 37 °C for 2 h and diluted to obtain a 10 mg/ml stock solution. Next, gefitinib (7– 8 mg; average particle size around 10 μm) or gefitinib-loaded PLGA microspheres (90– 100 mg, particle size 50–100 μm) were gently dispersed into 2 ml fibrinogen stock solution, mixed with a thin steel wire to avoid air bubbles. The fibrinogen solution (containing gefitinib or gefitinib-loaded PLGA microspheres) and the polymerization solution (Tris buffer saline (TBS) containing 7.5 mM calcium chloride and

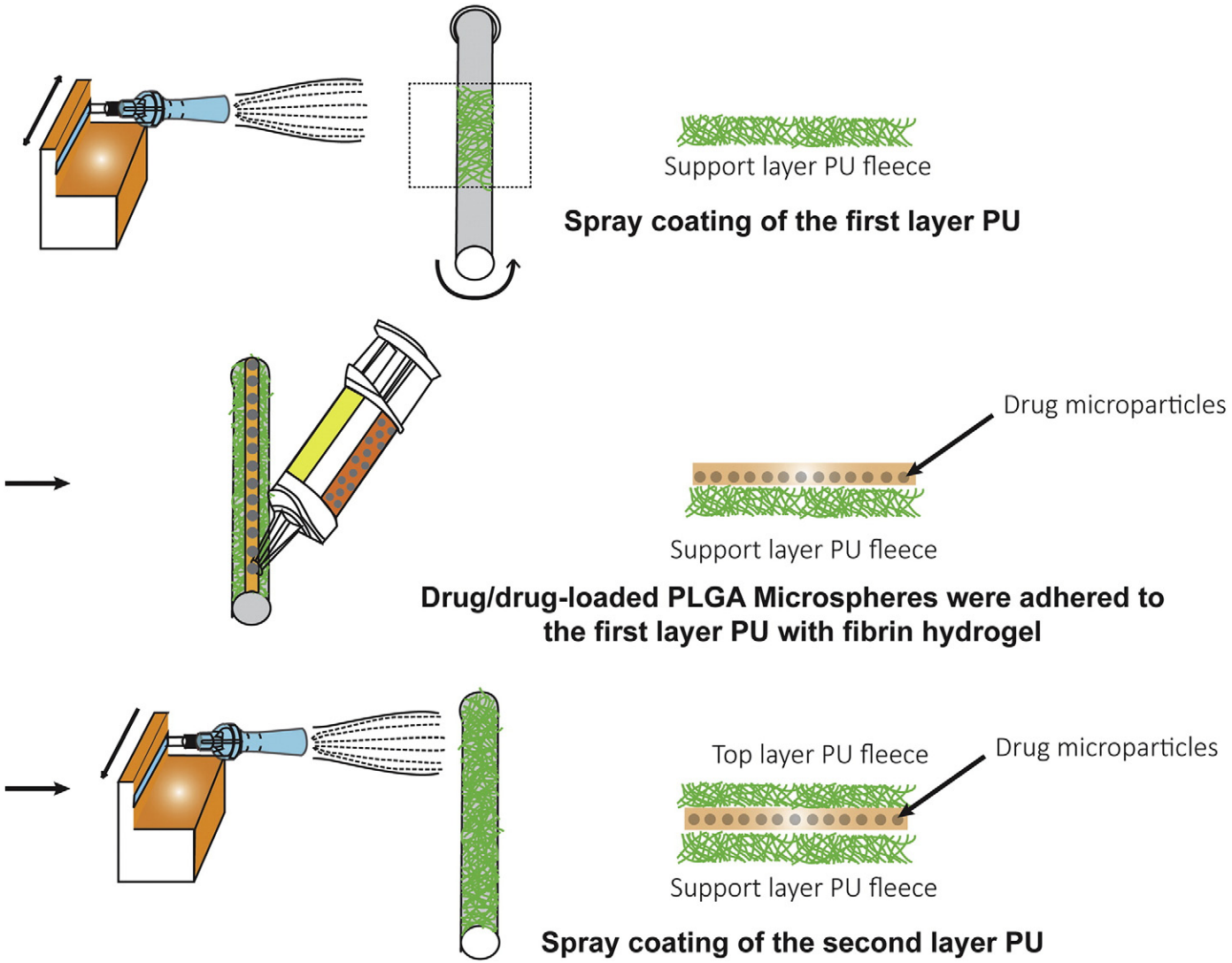

Fig. 2. Schematic drawing of the three-step spray-coating procedure. First, a PU support layer is sprayed and allowed to dry. Next, micronized drug particles or drug-loaded microspheres suspended in fibrin hydrogel are applied. After drying of the hydrogel, a top layer of PU is sprayed onto the construct.

6 U/ml thrombin) were filled separately in a double barrel syringe, and the solutions were subsequently applied into thin stripings onto the PU support layer. Gelation started immediately and was complete after 45 min. The spindles with the PU constructs were oven dried for 2 h at 30 °C.

2.3.3. Spray coating of the PU top layer

The spray coating procedure was the same as described in Section 2.3.1. By adjusting the duration of spray coating, PU top layers of different thicknesses were prepared. The spindle with the complete PU constructs were oven dried for 4 h at 30 °C. The PU constructs were then cut from the spindle and weighed.

2.4. Characterization of the PU constructs

2.4.1. Drug loading in the microspheres and PU constructs

Drug loading in microspheres and PU constructs was determined by HPLC after extraction with DMSO. Three milligrams of the gefitinib-loaded PLGA microspheres were accurately weighed and dissolved in 10 ml DMSO. The concentration of gefitinib in the DMSO solution was detected by HPLC as follows. The HPLC system consisted of Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module and a UV detector. Chromatographic data were acquired by using empower software 2. Analysis was carried out at 254 nm with Sunfire C_{18} column of 150 mm × 4.6 mm i.d., 5 μm dimensions (Waters) at ambient temperature. The mobile phase consisted of acetonitrile: MeOH: water: trifluoroacetic acid (0.3% *w*/*v*) at a ratio of 20:23:57 v/v/v that was set at a flow rate of 1.0 ml/min and with an injection volume of 50 μl. Gefitinib standards (1–150 μg/ml dissolved in DMSO) were used for calibration.

To determine the drug loading in the PU constructs, around 40 mg of PU constructs were immersed in 15 ml DMSO for 1, 2, or 3 days to extract the drug. The concentration of gefitinib in the DMSO solution was detected by the aforementioned HPLC method.

2.4.2. Residual solvent detection

Residual solvent of the PU constructs was determined by thermogravimetric analysis (TGA 2950 manufactured by TA Instrument Inc.). The PU constructs were cut into small rectangular pieces (3–10 mg) using a sharp razor blade. The samples were subsequently transferred into a platinum sample pan and heated from room temperature to 600 °C at a heating rate of 10 °C/min. The weight as function of temperature was recorded during the experiment using Universal Analysis 2000 data acquisition system. The content of residual solvent was calculated from the weight loss between room temperature and 120 °C.

2.4.3. Morphologies of gefitinib-loaded PU constructs

The morphologies of the PU constructs were examined by scanning electron microscopy (SEM), confocal laser-scanning microscopy (CLSM) and conventional fluorescence microscopy. Cross sections of the PU constructs (cut as small rectangle pieces (1 mm × 5 mm)) were covered by a fine platinum layer (6 nm) and glued onto 12 mm diameter aluminium specimen stubs (Agar Scientific Ltd., England) using double-side adhesive tape and analyzed by SEM (Phenom-World BV. Eindhoven, the Netherlands). Gefitinib crystals were analyzed as control. The thicknesses of the PU support and top layers were measured with the open source program ImageJ (NIH).

The homogenous dispersion of PLGA microspheres within the PU constructs was evaluated by CLSM (VK-9700, Keyence Corp.), with 0.1 nm resolution of vertical and horizontal measurement range, respectively. The laser power was monitored with a Gentec Electro-Optics SOLO 2 laser power meter. Microspheres were detected making use of the intrinsic fluorescence of Gefitinib. The excitation and emission wavelengths of gefitinib were 265 nm and 337 nm, respectively (Trummer et al., 2012).

Gefitinib-loaded PLGA microspheres and gefitinib micronized drug sandwiched in the PU constructs were also visualized by intrinsic fluorescence of gefitinib using a conventional fluorescence microscope (Keyence; BZ-9000) through DAPI filter. Samples were cut into small rectangle pieces and observed under 20× magnification.

2.4.4. Mechanical properties of PU constructs

Uniaxial tensile tests of the different PU sandwich constructs were carried out at room temperature using a Zwick biaxial testing machine (Zwick–Roell GmbH Group) combined with a calibrated video extensometer. Rectangular specimens of 25 mm long and 5 mm wide were cut from the PU constructs. Samples were tensile tested at a displacement rate of 10 mm/min using an initial gauge length of 10 mm.

2.5. In vitro drug release from gefitinib-loaded PU constructs

In vitro release of gefitinib release from the PU constructs was investigated by incubating the samples at 37 °C in PBS supplemented with 1% Tween 80 as incubation buffer to ensure sink condition for the release of gefitinib. The incubation buffer also containing 0.05% sodium azide to prevent bacterial growth. Samples of around 50 mg PU constructs were transferred into 15 ml incubation and incubated at 37 °C under constant shaking. Drug release was determined in 100 μl aliquots of release medium by HPLC analysis (as described in Section 2.4.1).

Cumulative gefitinib release from PU constructs was fitted (Graphpad Prism version 5) according to the (Korsmeyer-Peppas model: $Q = (M_t / M) = K_m t^n$; (Costa and Lobo, 2001). Q or M_t/M stands for fraction of drug released, t stands for the time since start of the release experiment, K_m is the release constant that includes structural properties of the drug and structural and geometrical properties of the release construct, and n is release exponent which is indicative of drug release mechanism.

To investigate the release direction of PU constructs loaded with gefitinib micronized drug were clamped in an in-house prepared diffusion cell system with two well-stirred compartments separated by the PU constructs. The volumes of the compartments were 4 ml each. The area of the PU films for the diffusion was 0.78 cm^2. Both compartments were filled with incubation buffer (PBS buffer pH 7.4 supplemented with 1% Tween 80 to maintain sink conditions for gefitinib). At indicated time points, 100 μl aliquots were collected from both compartments and replaced with the same volume of incubation buffer. Gefitinib released in the buffer was determined by HPLC as mentioned in Section 2.4.1.

Morphologies of gefitinib-loaded PLGA microspheres within PU constructs were analyzed by SEM (as described in Section 2.4.3). Samples of around 40 mg accurately weighed PU constructs were incubated at 37 °C under constant shaking. At different time points, PU constructs were collected and washed twice with reverse osmosis water. Afterwards, the samples were freeze dried for SEM detection.

3. Results and discussion

3.1. Sandwiched gefitinib/gefitinib-loaded PLGA microspheres in PU constructs

Gefitinib-loaded PLGA microspheres were prepared by a solvent evaporation technique and subsequently fractionated by wet-sieving using standardized square mesh sieves. Microspheres with size between 50 and 100 μm (gefitinib loading 7.3% w/w, drug encapsulation efficiency 82%) were chosen for embedding inside the PU constructs. Gefitinib/gefitinib-loaded PLGA microspheres were sandwiched in the PU constructs by a three-step method: firstly, a PU support layer was spray coated on the spindle; secondly, gefitinib/gefitinib-loaded PLGA microspheres were attached to the support layer by fibrin gel; thirdly, a PU top layer was spray coated.

Table 1 summarizes the characteristics of the formulations used in the study. From this table it is obvious that by adjusting the spray

Table 1
Characteristics of the PU sandwich constructs used in this study.

Samples	PU top layer						PU construct			
	Drug/MSP Deposit (mg)	Spray duration (min)	Spray volume (ml)	PU deposited (mg)	Calculated Top PU (mg/cm^2)	Measured thickness (μm)	Construct recovery[1] (%)	Constructs weight (mg)	Calculated LC[2] (w/w %)	Drug recovery (%)
Placebo[3]	–	2.0	4	480	3.5	–	98	1296	–	–
Gefitinib deposited (G)										
G1	7.9	–	–	–	–	–	98	833	0.79 ± 0.08	84 ± 2
G2	6.6	1.0	2	240	1.7	54	98	1065	0.54 ± 0.04	88 ± 3
G3	7.0	1.5	3	360	2.6	88	98	1180	0.52 ± 0.03	90 ± 1
G4	7.8	2.0	4	480	3.5	100	99	1308	0.50 ± 0.08	85 ± 2
G5	7.6	2.5	5	600	4.3	130	98	1414	0.49 ± 0.05	92 ± 3
G6	7.2	3.0	6	720	5.2	160	98	1533	0.43 ± 0.03	93 ± 1
G7	7.9	3.5	7	840	6.1	193	100	1656	0.41 ± 0.05	87 ± 2
Gefitinib-loaded PLGA microspheres deposited (GMSP)										
GMSP1	–	–	–	–	–	–	110	978	0.62 ± 0.05	83 ± 4
GMSP2	93.2	1.0	2	240	1.7	58	93	1059	0.55 ± 0.07	87 ± 3
GMSP3	105.5	1.5	3	360	2.6	91	94	1166	0.54 ± 0.03	85 ± 2
GMSP4[4]	97.1	2.0	4	480	3.5	106	102	1299	0.49 ± 0.05	90 ± 2
GMSP5	90.5	2.5	5	600	4.3	128	110	1428	0.41 ± 0.04	92 ± 1

The density of the PU support layer is 6.1 mg/cm^2, 840 mg/136 cm^2. G1-G7 refer to the PU constructs containing gefitinib micronized drug, GMSP1-GMSP5 refer to the PU constructs coating gefitinib-loaded PLGA microspheres. [1]Construct recoveries were calculated by dividing the mass of the final construct by the total weight of PU sprayed for support and top layers and the weight of MSP + fibrin. [2]Gefitinib loading content (LC) refers to the drug loading capacity as weight percentage of the total construct. [3]Placebo refers to the blank PU construct. [4]GMSP4 is the average data of three repeated samples: GMSP4a, GMSP4b, and GMSP4c.

coating duration of the PU top layer, the amount (spray coating duration × PU solution flow rate) of carbothane coated on the spindle can be precisely controlled. The flow rate of the PU solution sprayed was 2.0 ml/min. For all the samples, the PU support layers were prepared by spray coating 3.5 min of the PU solution on the spindle (so 7 ml of the solution, the theoretical PU deposited was 840 mg and the calculated support PU deposited on the spindle was 6.1 mg/cm^2). Porosities of similarly prepared drug-free PU constructs were around 79% (Chen et al., 2016a). The deposited PU of both support and top layer is not a closed uniform layer of PU but a flexible non-woven polymeric material with interstrand porosity. Differences in PU thicknesses will create materials with different tortuosity of the channels between polymer strands. Such channels can fill with water and hence can contribute to differences in drug dissolution (when considering water influx into the PU) and drug diffusion (drug efflux).

For all the samples, the thicknesses of the PU support layers were around 200 μm. By adjusting the spray duration (from 1.0–3.5 min), the thicknesses of the PU top layers of the samples can be precisely controlled (54–193 μm). The recoveries of the PU constructs, calculated as weight percentage of the sprayed amount of PU and the dry mass of applied fibrin and drug, ranged from 93 to 110%. The drug loading in samples of gefitinib/gefitinib-loaded PLGA microspheres incorporated PU constructs were from 0.41–0.79% and 0.41–0.62%, respectively. And the drug recoveries of gefitinib/gefitinib-loaded PLGA microspheres incorporated PU constructs were quite high, 85–93% and 83–92%, respectively. These data indicated that during the PU constructs preparation procedure, fibrin gel can firmly attach the gefitinib micronized drug or gefitinib-loaded PLGA microspheres on the PU support layers, so even after continuously rotating of the spindle, most of the materials were kept inside the constructs.

3.2. Residual solvent in PU constructs

The absence of residual solvents in the PU constructs was determined by thermographic analysis, i.e. by precisely measuring weight losses during heating of the samples. For the PU constructs, only minimal weight losses were observed below 120 °C, i.e. at temperatures at which evaporation of chloroform and water would result in a decrease for the total weight of the constructs. Weight losses were mainly observed above 120 °C, due to decomposition of the materials used to build the constructs (Fig. S1). It is unlikely that such weight losses represent the evaporation of DMSO (which was used during preparation of microspheres, together with dichloromethane) since DMSO is highly water soluble. Hence, this cosolvent will have been extracted quickly during solidification of the microparticles and the subsequent sieving steps to obtain uniformly sized microspheres.

3.3. SEM analysis of gefitinib and gefitinib-loaded PLGA microspheres embedded PU constructs

A critical concern of the system is that when spray coating the PU top layer, micronized gefitinib or gefitinib-loaded PLGA microspheres may partly dissolve in residual chloroform present in the polymer strands. Such interactions between PU and drug microparticles may affect drug eluting properties of the systems. In addition to the detection of residual solvent in the PU constructs (Section 3.2), several methods were furthermore used to investigate the morphologies of gefitinib micronized drug/gefitinib-loaded PLGA microspheres deposited PU constructs.

The typical SEM pictures of the gefitinib crystals (with average particle size of 10 μm measured by ImageJ), gefitinib deposited PU constructs and the PU support layer and top layer thicknesses of all the gefitinib deposited PU constructs (samples G1-G7) are shown in Fig. 3. The upper-left corner picture Fig. 3A shows gefitinib crystals under the SEM microscopy, while gefitinib crystals deposited in PU constructs without PU top layer (G1) and with the thickest PU top layer (G7) are shown in Fig. 3B and C. The gefitinib crystals sticking together formed bigger pieces inside the PU constructs. In Fig. 3B, the gefitinib crystals were firmly attached on the PU support layer even without the PU top layer which indicated that the fibrin gel played a positive role in attaching micronized drug. The fibrin gel structure cannot be observed under the SEM, since water inside the fibrin gel has evaporated after oven drying. Thicknesses of the PU top layers were measured by ImageJ analysis software and showed an increase in top-layer thicknesses along with extension of the spray coating duration (Fig. 3D).

Gefitinib-loaded PLGA microspheres were prepared by a solvent evaporation technique as described in Section 2.1 and the particles with size range of 50–100 μm were subsequently obtained through sieving. As shown in Fig. 4A, the solvent evaporation technique and the sieving procedure resulted in perfectly spherical particles with a

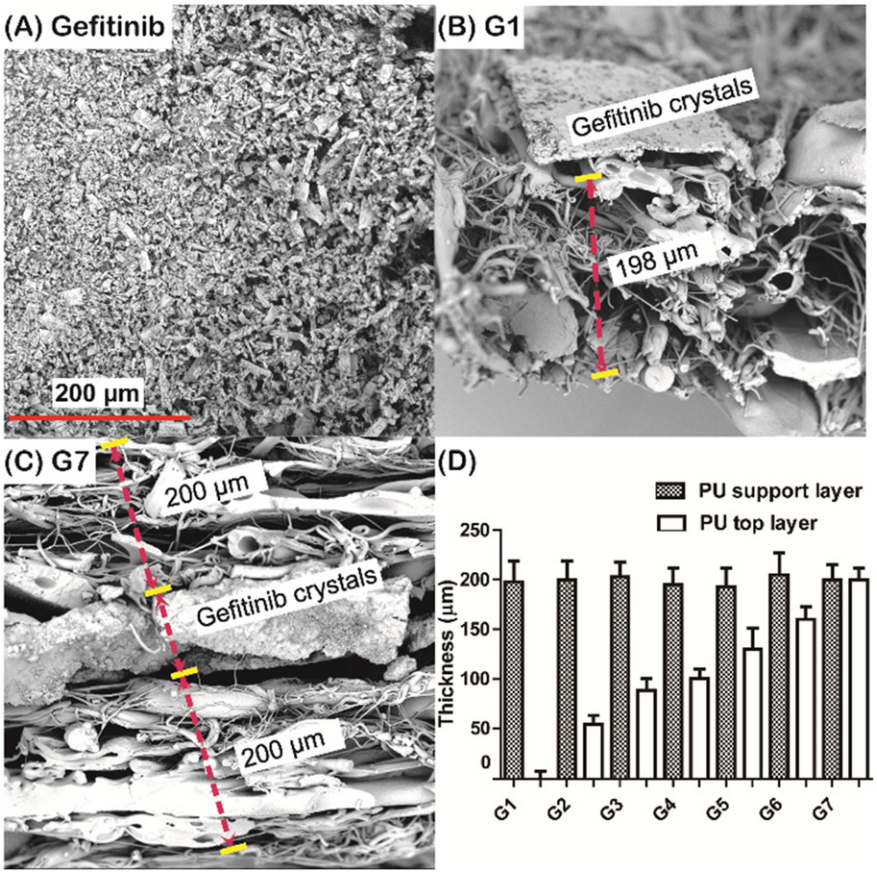

Fig. 3. SEM pictures of gefitinib crystals and gefitinib loaded PU constructs. (A): gefitinib micronized crystals; (B): PU construct G1 consisting of the PU support layer and gefitinib micronized crystals without the PU top layer; (C): PU construct G7 consisting of gefitinib micronized drug sandwiched between PU support layer and PU top layer; (D): thicknesses of the PU support and top layers of the gefitinib micronized drug sandwiched PU constructs were measured by software ImageJ and used to calculate the average thickness of 10 different measurements. The magnification is the same for all images (×600).

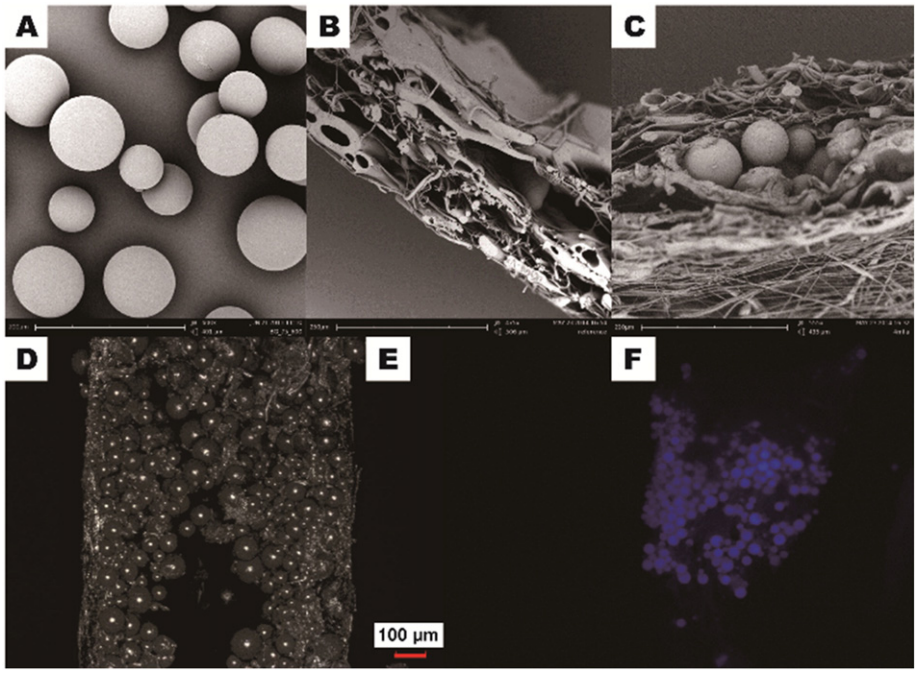

Fig. 4. Morphology of drug-loaded microspheres and PU constructs. (A): SEM pictures of gefitinib-loaded PLGA microspheres (with size range of 50–100 μm); (B): blank PU construct; (C): PU construct (sample GMSP4a) with gefitinib-loaded PLGA microspheres sandwiched between support and top PU layer; (D) laser scanning picture of sample GMSP4a (D); fluorescence microscopy pictures of blank PU construct (E) and sample GMSP4a (F).

smooth and non-porous surface. Fig. 4B shows the morphology of blank PU construct. Fig. 4C shows the morphology of the sample GM4, which has a PU support layer of ~200 μm and PU top layer of ~80 μm. All the other PU constructs which containing gefitinib-loaded PLGA microspheres (GM2, GM3, and GM5) have similar morphologies as Fig. 4C, the only difference is the thickness of the PU top layer. For all the samples, gefitinib-loaded PLGA microspheres can be clearly found in the PU constructs, the surfaces of the microspheres were even and smooth after embedding in PU. It means that during the spray coating of the PU top layer, the chloroform was sufficiently evaporated before reaching the spindle and did not dissolve gefitinib-loaded PLGA microspheres or affect the surface morphologies of the microspheres.

As shown in Fig. 4D, the embedding of microspheres in the PU constructs can also be observed clearly by confocal laser scanning microscopy (20 × magnification) which enabled the reconstruction of a larger segment of the PU construct than can be observed by SEM photomicrographs. The distribution of gefitinib microspheres within the PU was visualized by the autofluorescence of the gefitinib, which was also used to detect gefitinib microspheres by conventional fluorescence microscopy (Fig. 4F). The blank PU construct was set as a negative control (Fig. 4E).

3.4. Release of gefitinib from PU constructs

Gefitinib release was first studied from PU constructs loaded with gefitinib micronized crystals. As shown in Fig. 5A, sample G1, i.e. the sample without top layer, had all the gefitinib dissolved immediately which can be explained by the rapid dissolution of the drug exposed to the 15 ml of incubation medium. From samples G2 to G7, the drug release rate from the PU constructs decreases steadily with the increase of the thicknesses of the PU top layers.

Fitting of the release curves confirmed that gefitinib was released in a diffusion-based mechanism with release constants that decreased from 22.8 to 4.1 $\mu g/mm^2/t^{1/2}$ (fitted by the Korsmeyer-Peppas equation in Section 2.5, with n = 0.5) (see Table 2). A plausible explanation for this decrease in release rate is the increased tortuosity of water channels between the deposited nonwoven PU material with increasing thicknesses of the top layers.

For a drug-eluting stent coating, it makes sense that the drug has unidirectional release behavior, which means ideally the drug releases only through the PU top layer (to the tumor side of the bronchotracheal) but not the PU support layer (to the lumen of the bronchotracheal). To detect the drug release direction of the constructs, in-house prepared Franz-type diffusion cells were used (as shown in Supplementary data Fig. S2.)

We investigated the amounts of drug release from the two sides of the construct, reflecting drug release via the PU top layer and the PU support layer respectively. Profiles of drug release from different samples are shown in Fig. 6. In case of samples without the PU top layer (Fig. 6A), the drug dissolved into the release medium quickly (within 3 h), and there was no drug release detected from the PU support layer during the investigated period. Drug release from constructs G2 and G3 are shown in Fig. 6B and C. The drug release from the PU top layer exhibited a relatively high release rate during the investigated period (72% of drug release from the PU top layer of G2 after 8 days and 59% of drug release from the PU top layer of G3 after 12 days). In contrast, the drug release from the PU support layer was slower (11% of drug release from the PU support layer of G2 after 8 days and 21% of drug release from the PU support layer of G3 after 12 days). Fig. 6D shows the relative release rates of gefitinib in both compartments calculated from the Korsmeyer-Peppas model. During the investigation period, the average rate of drug release from the PU top layer were about 6 and 1.5 times higher than release via the PU support layer into the bottom compartment. These results demonstrate that the direction of drug transport is largely dependent on the relative thicknesses of the PU layers in the sandwich constructs.

Table 2
Release rates and correlation coefficients of drug release from PU constructs loaded with micronized gefitinib or gefitinib-PLGA microspheres.

Constructs with micronized gefitinib	Release rate constants ($\mu g/mm^2/d^{½}$)	Release exponent (n)	Correlation coefficient (r)
G1	22.78	0.50	1.0000
G2	11.21	0.50	0.9945
G3	9.16	0.50	0.9981
G4	6.10	0.50	0.9985
G5	5.04	0.50	0.9938
G6	4.42	0.50	0.9934
G7	4.09	0.50	0.9962

Constructs with gefitinib-PLGA microspheres	Release rate constant ($\%/d^n$)	Release exponent (n)	Correlation coefficient (r)
GMSP1	1.90	0.79	0.9785
GMSP2	1.51	0.82	0.9878
GMSP3	1.03	0.87	0.9857
GMSP4	0.52	0.91	0.9837
GMSP5	0.20	0.95	0.9854

3.5. In vitro drug release of gefitinib from microspheres deposited PU constructs

A pronounced extension of the release window was observed when gefitinib was formulated in microspheres as shown in Fig. 7A. Sustained gefitinib release was observed for all PU constructs loaded with gefitinib microspheres for over 7 months. As reported

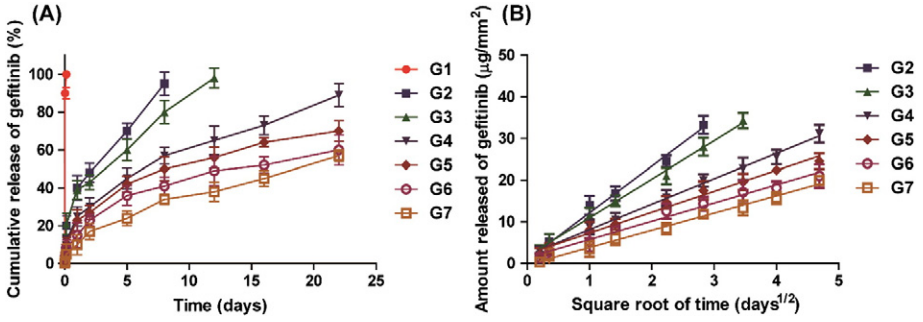

Fig. 5. Release rate of gefitinib from PU constructs loaded with micronized gefitinib with different thickness of PU top layers (G1–G7). (A): Cumulative release plotted versus linear time scale. (B) Cumulative gefitinib release plotted versus square root of time scale. Each sample was tested in duplicate.

previously, gefitinib was completely released from this type of drug-loaded PLGA microspheres (50–100 μm) in 80 days in a sigmoidal release profile that leveled off after the initial burst (<20% in the first week) and accelerated from day 30 until the end of the incubation at 3 months (Chen et al., 2016b). Gefitinib release from the microspheres deposited PU constructs showed a nearly linear release during the investigation period of 7 months. Burst release was largely reduced for all the PU constructs.

The slower and long-term sustained release observed from the PU constructs with gefitinib microspheres is most likely due to the additional diffusion layer surrounding the microspheres, or due to retarded drug release or matrix erosion as discussed in the next section. Fitting of the release curves by Korsmeyer-Peppas equation yielded values for the release exponent from 0.79–0.95, indicative of non-fickian or accelerated anomalous diffusion through the PU constructs (see Table 2).

When micronized drug was embedded directly in PU construct, drug release was controlled by diffusion as the rate limiting process. However, when drug was first loaded in microspheres then embedded in PU constructs, drug release from the microspheres became the rate limiting step. Several factors contribute to the drug release from microspheres, such as influx of water and dissolution of the drug within the particles, diffusion of gefitinib within the PLGA microparticles and erosion of the PLGA matrix (Chen et al., 2016b). Embedding of the particles within PU will furthermore change these parameters as will be shown below.

Similar as the drug release through different thickness of the PU layers, the drug release from the drug-loaded PLGA microspheres deposited inside PU constructs had the following trend: the thicker the PU top layer is, the slower the drug release from PU constructs. The schematic drawing of drug release from the PU constructs can be found in Fig. 7B. By controlling the thicknesses of PU layers, we can adjust the drug release direction and rate from PU constructs, which can be applied for reducing drug eluting to the bronchotracheal lumen side and correspondingly increasing drug release to the tumor site and enhancing anti-tumor effect of the stents.

Considering the unidirectional release of the drug for future stent coating, the coating material GMSP1 seems the best option according to the release curves. However, since such a material does not have a top layer that protects drug-loaded microspheres from abrasion during the implantation process, coatings with an additional PU layer that embeds the microparticles within the stent coating is preferred. Ideally, the top layer is thinner than the support layer, to promote directional release towards the tumor masses within the bronchotracheal wall.

3.6. In vitro degradation of gefitinib-loaded PLGA microspheres in PU constructs

As aforementioned, the drug release from the gefitinib-loaded PLGA microspheres deposited PU constructs was not only controlled by the drug diffusion from the microspheres and PU films, but also the degradation of the microspheres. The degradation behavior of the microspheres on top/inside of the PU films was studied by SEM as shown in Fig. 8.

Compared to the bare PLGA microspheres which completely degraded in around 3 months, the degradation rate of the microspheres on top/inside of the PU films was prolonged. After 2 months, nearly fully shaped microspheres can still be found in the samples, and after 3 month, fusion of the microspheres was found. Degradation of PLGA is governed by hydrolytic chain scission during which polymer chains are cleaved into oligomers and, finally, monomers (Alexis, 2005). Since PU-embedded microspheres are not in direct contact with the degradation medium, water first needs to diffuse through the PU fleece, which can be a rate limiting step in the degradation process when a closed PU coating is deposited on top of microspheres. It is difficult to draw

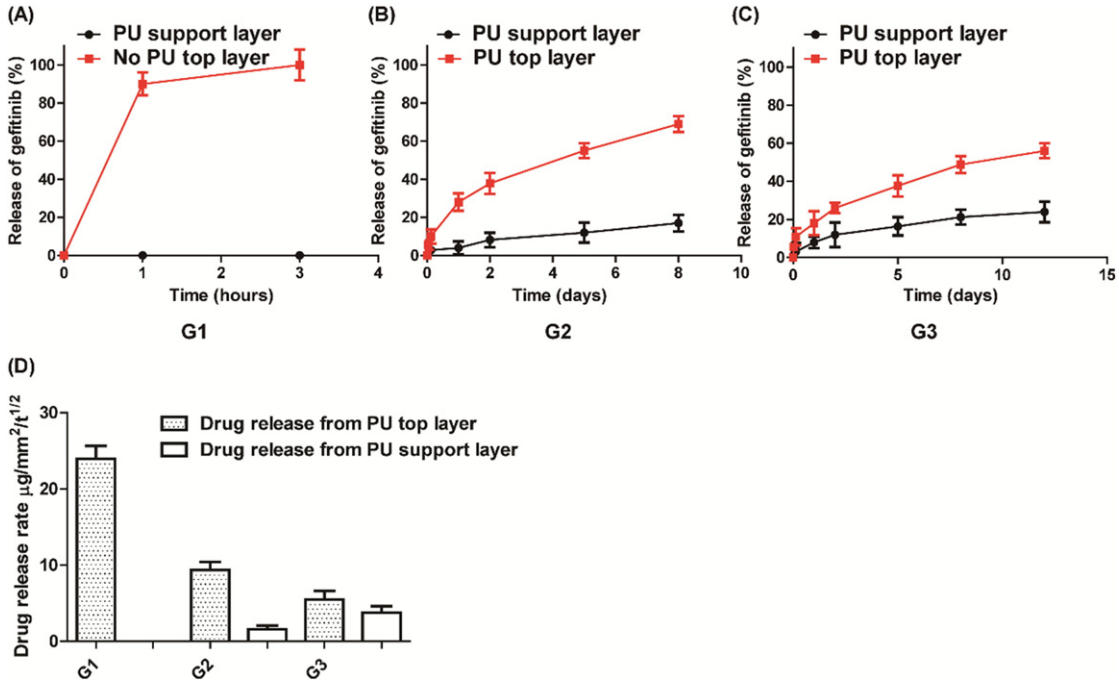

Fig. 6. Direction of drug release from PU constructs. Cumulative gefitinib release of PU constructs was measured in separate diffusion compartments connected to top or support layer. (A–C): gefitinib release from PU construct without top layer G1 (A), and from PU constructs different thicknesses of the PU top layer (B, C); the red lines refer to the drug release through the PU top layer and the black lines refer to the drug release through the PU support layer. (D): Relative gefitinib release via top and support layers. Each sample was tested in duplicate.

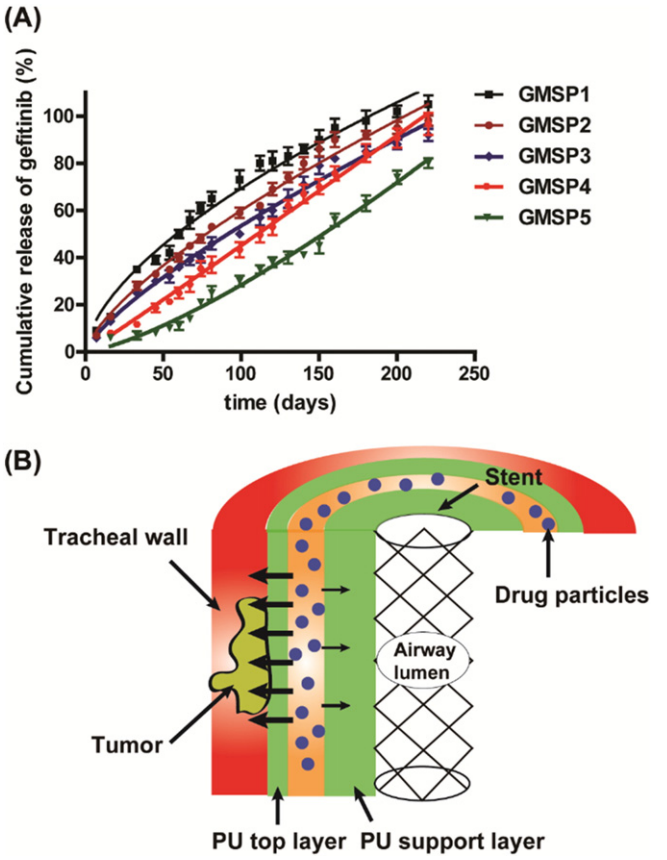

Fig. 7. Gefitinib release from PU constructs loaded with gefitinib-PLGA microspheres. (A): Cumulative drug release from PU constructs GMSP1-GMSP5. Curves were fitted by non-linear regression according to Korsmeyer-Peppas Equation (Section 2.5). Data are expressed as mean ± SD (n = 3). (B): Schematic representation of a bronchotracheal stent implanted in the upper airways after bronchoscope assisted surgical resection. Implantation of a stent will counteract stenosis of the upper airways. Gefitinib-PLGA microspheres embedded in the stent coating will provide long-term release of tumor suppressing drug.

a simple conclusion which determinant is rate-limiting in the degradation rate of the PLGA microspheres inside the PU constructs. The thickness of the PU top layer, the water permeability of the PU films, can all play a role of prolonging the degradation rate of the microspheres.

3.7. Mechanical properties of the PU constructs

Bronchotracheal stent coatings need to have proper flexibility to resist the rigors of compression and elongation required for deployment of the stent and subsequent peristaltic movements in situ (Guo et al., 2007). The tensible testing gives an indication of the strength and elasticity of the coating. PU is an elastic and tear-resistant polymer. However, drug-loaded PLGA microspheres deposited inside the PU constructs may have impact on its mechanical properties. As shown in Fig. 9, the PU constructs deposited with gefitinib-loaded microspheres GMSP4 (average strain-stress data of GMSP4a, GM4SPb, and GMSP4c) showed no significant differences compared to blank PU constructs. These results indicate that incorporation of microspheres (~8% w/w) in the PU didn't affect the mechanical property of the constructs. The modulus of elasticity of the samples were ~1 Mpa, which were quite low for all the samples (rubber 0.01–0.1 Gpa) (Fossen, 2010). It means on average the PU constructs had a low tensile strength, in another word, the samples had very high flexibility, which makes them advantageous for stents coating.

4. Conclusions

Gefitinib can be successfully loaded in PU sandwich structure as both micronized drug form and as gefitinib-loaded PLGA microspheres. Micronized drug particles embedded between two layers of PU constructs releases the drug in 1–3 weeks, while a support layer PU only (without the PU top layer) released all the drug in <1 day. Drug release profiles can be either extended by addition of a top PU layer or by embedding the drug in PLGA microspheres that are incorporated in the PU constructs.

Gefitinib release from the microspheres in PU constructs were controlled by both erosion of the microspheres inside the PU constructs

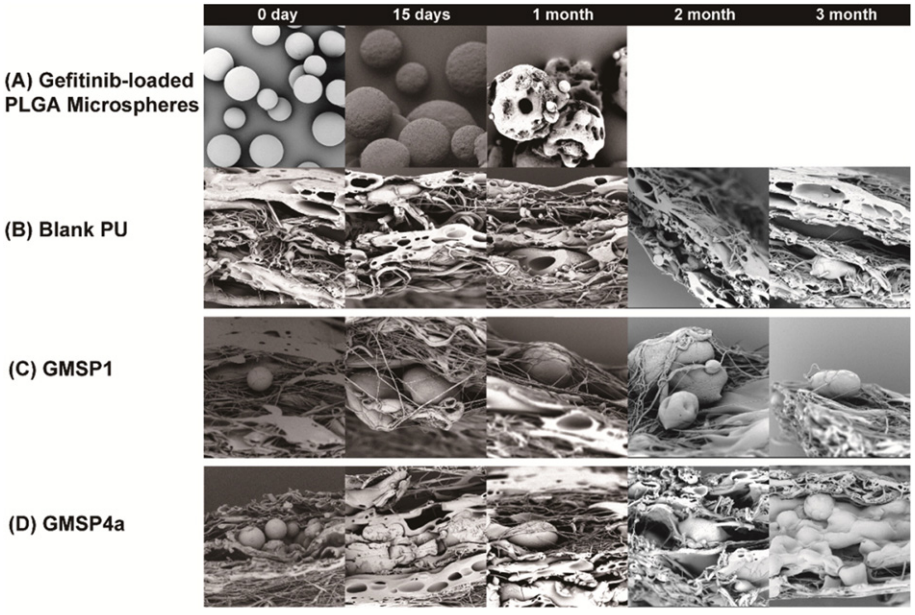

Fig. 8. In vitro degradation of gefitinib-PLGA microspheres embedded in PU constructs. SEM pictures of materials incubated at 37 °C in incubation buffer for indicated time periods. (A): gefitinib-loaded PLGA microspheres; (B): blank PU constructs; (C): GMSP1 construct, i.e. gefitinib-loaded PLGA microspheres deposited on PU support layer without PU top layer; (D): GMSP4a construct in which gefitinib-PLGA microspheres are embedded between support and top PU layer. Magnification is the same for all images (×600).

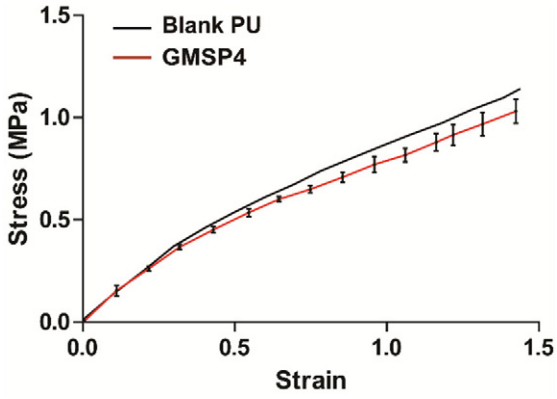

Fig. 9. Mechanical properties of PU constructs. Strain-stress curves of the blank PU construct (A) and GMSP4 (B). Average strain-stress curves were calculated from the curves of PU constructs GMSP4a, GMSP4b, GMSP4c.

and diffusion of gefitinib. Since the release rate of gefitinib from microspheres in PU constructs ≪ free gefitinib in PU constructs, the erosion of the microspheres is the rate controlling process. The embedding of microspheres didn't change the mechanical properties of the PU constructs and the gefitinib/gefitinib-loaded PLGA microspheres incorporated PU constructs are suitable for stent coating.

Acknowledgements

The authors would like to acknowledge the financial support from European Union's Seventh Framework Program (FP7/2007-2013 under grant agreement number NMP3-SL-2012-280915).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejps.2017.02.002.

References

Alexis, F., 2005. Factors affecting the degradation and drug-release mechanism of poly (lactic acid) and poly [(lactic acid)-*co*-(glycolic acid)]. Polym. Int. 54, 36–46.
Barker, A.J., Gibson, K.H., Grundy, W., Godfrey, A.A., Barlow, J.J., Healy, M.P., Woodburn, J.R., Ashton, S.E., Curry, B.J., Scarlett, L., 2001. Studies leading to the identification of ZD1839 (Iressa™): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer. Bioorg. Med. Chem. Lett. 11, 1911–1914.
Bayer, O., 1947. Das di-isocyanat-polyadditionsverfahren (polyurethane). Angewandte 59, 257–272.
Breen, A., O'Brien, T., Pandit, A., 2009. Fibrin as a delivery system for therapeutic drugs and biomolecules. Tissue Eng. B Rev. 15, 201–214.
Cederholm Williams, S., Marshall, J., Velada, J., Hollingsbee, D., 2002. Fibrin Polymer Structure (Patent No. 6, 462,018).
Chen, M.S., John, J.M., Chew, D.P., Lee, D.S., Ellis, S.G., Bhatt, D.L., 2006. Bare metal stent restenosis is not a benign clinical entity. Am. Heart J. 151, 1260–1264.
Chen, Q., Liang, S., Thouas, G.A., 2013. Elastomeric biomaterials for tissue engineering. Prog. Polym. Sci. 38, 584–671.
Chen, W., Habraken, T.C., Hennink, W.E., Kok, R.J., 2015. Polymer-free drug-eluting stents: an overview of coating strategies and comparison with polymer-coated drug-eluting stents. Bioconjug. Chem. 26, 1277–1288.
Chen, W., Clauser, J., Thiebes, A.L., McGrath, D.J., McHugh, P.E., Steinseifer, U., Jockenhoevel, S., Hennink, W.E., Kok, R.J., 2016a. Selection and fabrication of a non-woven polycarbonate urethane cover for a tissue engineered airway stent. Int. J. Pharm. 514, 255–262.
Chen, W., Palazzo, A., Hennink, W.E., Kok, R.J., 2016b. The effect of particle size on drug loading and release kinetics of gefitinib-loaded PLGA microspheres. Mol. Pharm.
Cherng, J.Y., Hou, T.Y., Shih, M.F., Talsma, H., Hennink, W.E., 2013. Polyurethane-based drug delivery systems. Int. J. Pharm. 450, 145–162.
Costa, P., Lobo, J.M.S., 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13, 123–133.
Fossen, H., 2010. Structural Geology. Cambridge University Press.
Freiberg, S., Zhu, X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1–18.
Guo, Q., Guo, S., Wang, Z., 2007. A type of esophageal stent coating composed of one 5-fluorouracil-containing EVA layer and one drug-free protective layer: in vitro release, permeation and mechanical properties. J. Control. Release 118, 318–324.
Herth, F.J., Eberhardt, R., 2016. Airway stent: what is new and what should be discarded. Curr. Opin. Pulm. Med. 22, 252–256.
Hohenforst-Schmidt, W., Zarogoulidis, P., Pitsiou, G., Linsmeier, B., Tsavlis, D., Kioumis, I., Papadaki, E., Freitag, L., Tsiouda, T., Turner, J.F., 2016. Drug eluting stents for malignant airway obstruction: a critical review of the literature. J. Cancer 7, 377.
Hu, T., Yang, J., Cui, K., Rao, Q., Yin, T., Tan, L., Zhang, Y., Li, Z., Wang, G., 2015. Controlled slow-release drug-eluting stents for the prevention of coronary restenosis: recent progress and future prospects. ACS Appl. Mater. Interfaces 7, 11695–11712.
Kwon, H.J., Park, S., 2014. Local delivery of antiproliferative agents via stents. Polymers 6, 755–775.
Lamba, N.M., Woodhouse, K.A., Cooper, S.L., 1997. Polyurethanes in Biomedical Applications. CRC press.
Lei, L., Liu, X., Guo, S., Tang, M., Cheng, L., Tian, L., 2010. 5-Fluorouracil-loaded multilayered films for drug controlled releasing stent application: drug release, microstructure, and ex vivo permeation behaviors. J. Control. Release 146, 45–53.
Lei, L., Guo, S.-R., Chen, W.-L., Rong, H.-J., Lu, F., 2011. Stents as a platform for drug delivery. Expert Opin. Drug Deliv. 8, 813–831.
Nadzeyka, I., Gabler, C., Erarslan, D., Safi, Y., Steinseifer, U., 2014. Manufacturing of biocompatible nonwoven structures by using spray atomization of dissolved polymers. Polym. Eng. Sci. 54, 867–873.
Puel, J., Joffre, F., Rousseau, H., Guermonprez, J., Lancelin, B., Morice, M., Valeix, B., Imbert, C., Bounhoure, J., 1987. Endo-prothèses coronariennes auto-expansives dans la prévention des resténoses après angioplastie transluminale: étude clinique préliminaire. Arch. Mal. Coeur Vaiss. 80, 1311–1312.
Puranik, A.S., Dawson, E.R., Peppas, N.A., 2013. Recent advances in drug eluting stents. Int. J. Pharm. 441, 665–679.
Rask, F., Mihic, A., Reis, L., Dallabrida, S.M., Ismail, N.S., Sider, K., Simmons, C.A., Rupnick, M.A., Weisel, R.D., Li, R.-K., 2010. Hydrogels modified with QHREDGS peptide support cardiomyocyte survival in vitro and after sub-cutaneous implantation. Soft Matter 6, 5089–5099.
Reck, M., Heigener, D.F., Mok, T., Soria, J.C., Rabe, K.F., 2013. Management of non-small-cell lung cancer: recent developments. Lancet 382, 709–719.
Roguin, A., 2011. Stent: the man and word behind the coronary metal prosthesis. Circ. Cardiovasc. Interv. 4, 206–209.
Seo, E.H., Na, K., 2014. Polyurethane membrane with porous surface for controlled drug release in drug eluting stent. Biomater. Res. 18, 1–5.
Seo, J., Lee, J., Na, K., 2016. Polymeric materials for drug release system in drug eluting stents. J. Pharm. Investig. 46, 317–324.
Shaffer, J.P., Allen, J.N., 1998. The use of expandable metal stents to facilitate extubation in patients with large airway obstruction. Chest 114, 1378–1382.
Simmons, A., Padsalgikar, A.D., Ferris, L.M., Poole Warren, L.A., 2008. Biostability and biological performance of a PDMS-based polyurethane for controlled drug release. Biomaterials 29, 2987–2995.
Sun, D., Zheng, Y., Yin, T., Tang, C., Yu, Q., Wang, G., 2014. Coronary drug-eluting stents: from design optimization to newer strategies. J. Biomed. Mater. Res. A 102, 1625–1640.
Thiebes, A.L., Kelly, N., Sweeney, C.A., McGrath, D.J., Clauser, J., Kurtenbach, K., Gesche, V.N., Chen, W., Kok, R.J., Steinseifer, U., 2016. PulmoStent: in vitro to in vivo evaluation of a tissue engineered endobronchial stent. Ann. Biomed. Eng. 1–11.
Trummer, B.J., Iyer, V., Balu-Iyer, S.V., O'Connor, R., Straubinger, R.M., 2012. Physicochemical properties of epidermal growth factor receptor inhibitors and development of a nanoliposomal formulation of gefitinib. J. Pharm. Sci. 101, 2763–2776.
Wakeling, A.E., Guy, S.P., Woodburn, J.R., Ashton, S.E., Curry, B.J., Barker, A.J., Gibson, K.H., 2002. ZD1839 (Iressa) an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res. 62, 5749–5754.
Zdrahala, R.J., Zdrahala, I.J., 1999. Biomedical applications of polyurethanes: a review of past promises, present realities, and a vibrant future. J. Biomater. Appl. 14, 67–90.