Novel farnesylthiosalicylate (FTS)-eluting composite structures

Amir Kraitzer, Yoel Kloog, Meital Zilberman

ARTICLE INFO

Article history:
Received 18 November 2008
Received in revised form 19 February 2009
Accepted 9 March 2009
Available online 21 March 2009

Keywords:
Farnesylthiosalicylate (FTS)
Drug-eluting stents
Drug-eluting fibers
Stent coatings
Degradation

ABSTRACT

Farnesylthiosalicylate (FTS) is a new specific nontoxic drug with a mild hydrophobic nature, which acts as a Ras antagonist and can therefore be used for stent applications as well as for local cancer treatment. FTS-loaded biodegradable core/shell fiber structures were developed and studied in order to investigate the FTS release mechanism. These structures were composed of a polyglyconate core and a porous poly(ε-lactic-glycolic acid) shell loaded with FTS, prepared using freeze drying of inverted emulsions. The effects of the emulsion’s composition (formulation) and process kinetics on the FTS release from the coatings were studied with reference to the shell morphology and degradation profile. The FTS release profiles exhibited a burst effect accompanied by a release rate which decreased with time and lasted for 15–40 days. The process was found to affect the drug release profile via two routes: (1) Direct, through water uptake and swelling of the structure, leading to a FTS burst release. Degradation of the host polymer affects the FTS release rate at a later stage. (2) Indirect effect of the microstructure on the release profile, which occurs via an emulsion stability mechanism. The copolymer composition is the most important parameter affecting the release behavior in our system. Other parameters, including polymer content, O:A phase ratio and homogenization rate exhibited only minor effects on the FTS release profile. The controlled release of the new drug FTS is reported here for the first time.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Drug-eluting stents (DES) significantly reduce the incidence of in-stent restenosis (ISR), which was once considered a major adverse outcome of percutaneous coronary stent implantation. Localized release of antiproliferative drugs interferes with the pathological proliferation of vascular smooth muscle cells (VSMC) which is the main cause of in-stent restenosis (Heldman et al., 2001). Drug uptake into the vessel wall occurs by passive diffusion and convection and is facilitated by the hydrophobic nature of these antiproliferative drugs that establish substantial partitioning and spatial gradients across the tissue (Creel et al., 2000; Jackson et al., 2004). In the USA, use of drug-eluting stents has increased from 19.7% at the time of their approval to 78.2% of percutaneous procedures at the end of 2004 (Rao et al., 2006). Today, the most extensively studied DES are the commercially available TAXUS™ (Boston Scientific, paclitaxel-eluting stent) and Cypher™ (Cordis, sirolimus-eluting stent). However, drug-eluting stents are associated with increased rates of late stent thrombosis (LST) (Lagerqvist et al., 2007) and hypersensitivity reactions (Virmani et al., 2004), which are serious low-frequency life-threatening complications.

Three factors affect the efficacy and safety of current DES: the stent platform, the drug release matrix, and the type of drug released. Cypher and Taxus are based on a 316L stainless steel strut stent platform. The coating of the Cypher and Taxus stents is composed of durable dense polymers (Moreno and Macaya, 2005), which may trigger hypersensitivity reactions as described in a case study by Virmani et al. (2004). The stent coating should also have good mechanical properties in terms of flexibility and long-lasting adherence to the stent surface (Rogers, 2004), especially when the stent is expanded to the required size during surgery. This expansion can seriously affect the release of the drug from the polymer, or worse, can embolize the polymer (Finkelstein et al., 2003).

The drug release kinetics of current DES is far from optimal in terms of safety and efficacy, despite the accumulated knowledge supporting its importance. The hydrophobic nature of the released antiproliferative drugs and the non-degradable nature of the coating matrix along with the small thickness of current DES coatings offer relatively poor control over the release period (Wang et al., 2006) and a relatively low drug load. Cypher contains 70–300 μg sirolimus (140 μg/mm²) and 80% of the drug is released within 28 days after stent implantation (Venkatraman and Boey, 2007). Taxus contains 50–200 μg (1 μg/mm²) paclitaxel, where ~2 μg
are released within 15 days (Venkatraman and Boey, 2007) and 92.5% of the drug remains in the matrix for a long period (Serruys et al., 2005). The drug release kinetics from these stents may be altered via the drug/polymer ratio (Halkin and Stone, 2004), coating thickness, and/or coating a drug-free top layer onto the drug reservoir layer, in order to create the slow-release formulation as demonstrated for the Cypher sirolimus-eluting stent (Venkatraman and Boey, 2007). Drachman et al. (2000) studied bioreosorbable poly(lactide-co-ε-caprolactone) coated metal stents that release paclitaxel and reported that the in vitro release profile followed first order kinetics with almost 90% of the drug being released within about 2 months, and a burst effect of about 36%. Finkelstein et al. (2003) used alternating layers of 50/50 PLGA and PLLA inside metal struts to control the paclitaxel release profile and burst effect. More recent studies changed the hydrophobic/hydrophilic balance in the polymer matrices in order to affect the release kinetics from the stents (Breitenbach et al., 2000; Westedt et al., 2006).

The released drug also affects the efficacy and safety of DES. Sirolimus is a nonspecific immunosuppressive agent that inhibits cell cycle progression at the early stage (leading to reversion of the cell to the quiescent state) (Venkatraman and Boey, 2007). Thus, sirolimus-coated DES prevent not only VSMC hyperplasia but also re-endothelialization (Li et al., 2006), or may cause endothelial dysfunction (Hofma et al., 2006) on the inner side of the metal stent. Paclitaxel is a microtubule stabilizing agent that inhibits cell cycle progression at the late stage (leading to cell death and apoptosis) (Venkatraman and Boey, 2007), and has a low therapeutic index (Fonseca et al., 2002). It is therefore very effective in the treatment of nonintimal hyperplasia, which is known as the main cause of restenosis (Feng and Huang, 2001). However, it may also be very toxic.

Farnesylthiosalicylate (FTS, Salirasib) is a new rather specific nontoxic drug which was recently developed at the Tel-Aviv University (Marom et al., 1995). Its chemical structure is presented in Scheme 1. FTS acts as a Ras antagonist (George et al., 2004; Drachman et al., 2000) and has a mild hydrophobic nature. In its active form (GTP-bound), FTS promotes enhanced cell proliferation, tumor cell resistance to drug-induced cell death, enhanced migration and invasion. Ras is therefore considered an important target for cancer therapy as well as for therapy of other proliferation diseases, including restenosis. The apparent selectivity of FTS towards active (GTP-bound) Ras and the absence of toxic or adverse side effects was proven in animal models (George et al., 2004) and in humans (Concordia Pharmaceuticals, Inc., Ft. Lauderdale, FL). In the rat carotid artery injury model, which serves as a model for restenosis, FTS was found to be a potent inhibitor of intimal thickening while not interfering with endothelial proliferation (George et al., 2004). The incorporation of the new drug, FTS, in a stent coating may overcome the incomplete healing and lack of endothelial coverage associated with current drug-eluting stents.

Current drug-eluting biodegradable or biostable stent coatings that contain antiproliferative drugs exhibit serious side effects and are consequently far from optimal in terms of their drug loading and release profiles. These coatings cannot carry sufficient amounts of drug because of the trade-off between the mechan-
2.2. Preparation of core/shell fiber structures

2.2.1. Fiber surface treatment

The sutures were surface-treated in order to enhance the adhesion between the core fiber and the coating. The polyglyconate fibers were slightly stretched on special holders and dipped in 1,1,1,3,3,3-hexafluoro-2-propanol (hexafluorisopropanol) for 40 s. The fibers were then washed with ethanol and dried at room temperature.

2.2.2. Emulsion formation

A known amount of 50/50 PDLGA was dissolved in chloroform to form an organic solution and FTS was added to the solution. Double-distilled water was then poured into the organic phase (in a scintillation vial) and homogenization of the emulsion was performed using a homogenizer (Polytron PT3100 Kinematica, 12 mm rotor) operating at 16,500 rpm (medium rate) for 2 min, for most investigated samples. As a reference sample we chose an emulsion formulation containing 12.5% (w/v) polymer in the organic solution, 2% (w/w) FTS (relative to the polymer load), and an organic to aqueous (O:A) phase ratio of 4:1 (v/v). Other formulations included 20% (w/v) polymer, 1% and 4% (w/w) FTS, O:A phase ratios of 2:1 and 8:1 and copolymer composition of 75/25 PDLGA. Certain samples were prepared using homogenization rates of 8500 rpm (low rate) or 22,500 rpm (high rate) in order to investigate the effect of processing kinetics on the porous shell structure.

2.2.3. Core/shell fiber structure formation

The treated core polyglyconate fibers were dip-coated (while placed on holders) in fresh emulsions and then frozen immediately in a liquid nitrogen bath. The holders + samples were then placed in a pre-cooled (−105 °C) freeze dryer (Virvit 100 equipped with a nitrogen trap) capable of working with organic solvents (freezing temperature of the condenser was approximately −105 °C) and freeze dried in order to preserve the temporal state of the emulsion in a solid form. The shell's microstructure thus reflects the emulsion's stability. The freeze dryer chamber pressure was reduced to 100 mTorr while the temperature remained constant (−105 °C) in order to sublimate the water and solvents. Room temperature was then slowly restored in order to evaporate residual solvent vapors. The samples were then stored in desiccators until use.

2.3. Morphological characterization

The morphology of the composite core/shell fiber structures (cryogenically fractured surfaces) was observed using a Jeol JSM-6300 scanning electron microscope (SEM) using an accelerating voltage of 5 kV. The SEM samples were Au sputtered prior to observation. The mean pore diameter of the observed morphologies was analyzed using the Sigma Scan Pro software.

All quantitative measurements were summarized as means ± standard deviations, unless otherwise specified. Comparisons of the mean pore diameter were carried out for the microstructure analysis using the unpaired Student's t-test for two group comparisons, or ANOVA (post hoc Tukey-Kramer) for three group comparisons. SPSS was used for all statistical calculations. Statistical significance was determined at p < 0.05.

In vitro morphology of wet shell structures was characterized using an environmental SEM (Quanta 200 FEG ESEM) using an accelerating voltage of 10 kV in a pressure of 4.5 Torr. Fractographs were taken at three time points while the specimens were maintained in double-distilled water outside the ESEM. The specimens were fixed to a special base, and initial coordinates were recorded so as to enable good return to these coordinates.

2.4. In vitro FTS release studies

The composite core/shell fiber structures were immersed in phosphate buffered saline (PBS) at 37 °C and pH = 7.4 for 35 days, in triplicates, in order to determine the release kinetics from the FTS-loaded composite structures. The release studies were conducted in closed glass tubes containing 3 ml PBS medium, using a horizontal bath shaker operated at a constant rate of 130 rpm. The medium was removed (completely) periodically, at certain sampling times, and measured as described in Section 2.5. Fresh medium was then introduced. At the end of the experiment the fibers were immersed in methylene chloride and the residual amount of drug was measured. This experiment enabled simulating conditions similar to those which exist in a blood vessel.

2.5. FTS extraction procedure

FTS extraction from the medium was performed as follows: the 3 ml PBS/FTS medium was completely removed at each time point and placed in a scintillation vial. Three milliliters acetonitrile and 1 ml methylene chloride were added and methylene chloride evaporation was performed under a nitrogen stream (99.999% grade). Medium (50/50, v/v acetonitrile/PBS) was added up to 4 ml in each test tube. The FTS concentration was then estimated using HPLC. An extraction factor was used for correction. Known weights of FTS were dissolved in 3 ml acetonitrile and 3 ml PBS and 1 ml methylene chloride was added. The known concentrations were subjected to the same extraction procedure as the unknown concentrations in order to determine the efficiency of the extraction procedure. The recovery efficiency of the method was 88.4% and the value of the measured drug was corrected accordingly.

The FTS content of the medium samples was determined using Jasco High Performance Liquid Chromatography (HPLC) with a UV 2075 plus detector and a reverse phase column (ACE 5 C18, inner diameter = 4.6 mm, length = 250 mm), equipped with a column guard, and was kept at room temperature (25 °C). The mobile phase consisted of a mixture of acetonitrile and phosphate buffer (30 mM, pH = 4.5) at a ratio of 70/30 (v/v), respectively, at a flow rate of 1 ml/min with a quaternary gradient pump (PU 2089 plus) without gradient. Hundred microliter samples were injected with an autosampler (AS 2057 Plus). The column effluent was eluted for 15 min and detected at 322 nm. The area of each eluted peak was integrated using the EZstart software version 3.1.7.

2.6. Residual drug recovery from the composite fibers

On the final day of the in vitro trial, residual FTS from the composite fibers was measured as follows: the fibers were placed in 1 ml methylene chloride for 10 min and the coating shell was dissolved. 6 ml of a 50/50 acetonitrile/water solution were then added and the polyglyconate core was removed. Methylene chloride evaporation was performed under a nitrogen (99.999%) stream. Medium (50/50, v/v acetonitrile/water) was added until 4 ml in each test tube and the FTS concentration was estimated by HPLC using the same method as above. An overall calibration curve for both HPLC and method recovery was calculated using known amounts of FTS under the same conditions.

The cumulative release profiles were determined relative to the initial amount of FTS in the composite fibers (quantity released during the incubation period + the residue remaining in the fibers). All experiments were performed in triplicates. Results are presented as means ± standard deviations. The effects of the emulsion's formulation on the release profile were studied by examining the following parameters: polymer content in the organic phase (% w/w), drug content relative to polymer content (% w/w), organic-aqueous
(O:A) phase ratio and copolymer composition. The effect of the process kinetics (homogenization rate) on the release profile was also studied.

2.7. Encapsulation efficiency calculations

The encapsulation efficiency (EE) of the fibers was calculated as the actual amount \( M_t \) of drug measured in each fiber divided by the theoretical amount of drug \( M_0 \) encapsulated during the fabrication process, presented in percentage as shown in Eq. (1). The actual amount of drug encapsulated within each fiber is the accumulated amount of FTS released at each measurement point of the trial plus the residual amount measured on day 35. The theoretical amount of FTS is the formulation drug concentration multiplied by the weight of the coating. The weight of the coating is the difference between the coated fiber (weighed at the beginning of the \textit{in vitro} trial) and the bare fiber (weighed at the end of the trial, denuded of the coating by immersion in methylene chloride). The results are presented as means ± standard deviations \((n = 3)\).

\[
EE = \frac{M_t}{M_0} \times 100
\]

(1)

2.8. FTS chemical stability

Two milligrams of FTS were placed in 3 ml PBS at 37 °C for 100 days in order to determine its stability throughout the release experiment. The closed scintillation vials containing the FTS in PBS were placed in a horizontal bath shaker operated at a constant rate of 130 rpm. The FTS content of a single vial was measured at each point of time.

FTS was extracted as follows: 3 ml methylene chloride were added to the 3 ml PBS/FTS mixture and stirred for 10 min in order to dissolve the drug. One milliliter was carefully removed from the bottom of the vial (the organic phase) using a pipette and placed in a new vial. Five milliliters of 50/50 acetonitrile/double-distilled water (the medium) were added to the vial and then evaporated under nitrogen conditions. The content of each vial was transferred to a test tube and diluted to 20 ml. The FTS content of the medium samples was determined using HPLC, as described above.

2.9. In vitro degradation of the porous PDLGA structure

Porous 50/50 PDLGA and 75/25 PDLGA film structures were fabricated as described earlier, in the paragraph about core/shell fiber structure formation but without drug. The inverted emulsion was prepared as described earlier, poured into an aluminum plate, quenched in liquid nitrogen, and freeze dried. Each sample (three repetitions), approximately 1 cm², was incubated in 40 ml phosphate buffered saline (PBS) containing 0.05% (v/v) sodium azide (as preservative) at 37 °C under static conditions for 5 weeks. PBS was added when the pH was out of range (between 7 and 8) or when the PBS volume dropped below 15 ml. The samples were taken out at weekly intervals, filtered using a Whatman size 2 filter paper and dried in a vacuum oven (35 °C for 2 h).

Mass loss was measured using a Mettler-Toledo microbalance. The normalized mass loss was calculated by comparing the mass at a given time point \( w_i \) with the initial mass \( w_0 \) as shown in Eq. (2). The results are presented as means ± standard deviations \((n = 3)\).

\[
\text{Normalized weight (%) } = \frac{w_i}{w_0} \times 100
\]

(2)

2.10. In vitro weight loss profile of the porous PDLGA structure

Porous 50/50 PDLGA and 75/25 film structures were fabricated as described earlier, in the paragraph about the \textit{in vitro} degradation study. Each film sample was cut into pieces of approximately 1 cm² and then incubated in 15 ml double-distilled water at 37 °C under static conditions. Samples (triplicates) were taken out at weekly intervals, filtered using a 70 μm filtration paper and dried in a vacuum oven (35 °C for 2 h).

2.11. Measurements of water uptake

Porous 50/50 PDLGA film structures were fabricated as described earlier, in the paragraph about the \textit{in vitro} degradation study. Each film sample was cut into pieces of approximately 1 cm² and then incubated in 15 ml double-distilled water at 37 °C under static conditions. Samples (triplicates) were taken out periodically and immediately subjected to measurement of wet weight, after surface water was removed with a clean-wipe tissue. Water uptake, i.e. adsorption and absorption of each sample during the swelling period, was determined according to Eq. (3) \((w_i - w_0) / w_0 \times 100\) (w is the wet weight at each time point and \( w_0 \) is the dry weight measured before the incubation):

\[
\text{Water uptake (%) } = \frac{w_i - w_0}{w_0} \times 100
\]

(3)

2.12. Measurements of tensile mechanical properties and their degradation

Core shell fiber structures were fabricated as described above (in the paragraph about core/shell fiber structure formation but without drug). The fibers’ tensile mechanical properties were measured at room temperature, under unidirectional tension at a rate of 50 mm/min, using a 5544 Instron uniaxial machine. Three fiber types (Maxon sutures, surface-treated fibers and coated fibers (without the drug), \( n = 5 \) for each sample, 14 cm in length) were wrapped around a thick paper and inserted between the jigs. The tensile strength was defined as the maximum strength in the stress–strain curve. The maximal strain was defined as the breaking strain. Young’s modulus was defined as the slope of the stress–strain curve in the elastic (linear) region. Means ± standard deviations are presented.

In order to evaluate the degradation of mechanical properties, samples were immersed in 14 ml PBS filled tubes for 84 days. Each tube contained five specimens of 14 cm coated fibers. The pH was maintained between 7.3 and 7.5 and the medium was changed when the pH was out of the range. Each week a single tube was retrieved and the fibers were dried using a vacuum oven (35 °C for 1.5 h) and kept in a desiccator. Each specimen’s diameter was measured using a caliper and a tensile test was carried out using an Instron machine as described above.
Table 1
The examined specimens’ shell structural characteristics and encapsulation efficiency.

<table>
<thead>
<tr>
<th>Process parameters</th>
<th>Mean pore size ± SD (nm)</th>
<th>Mean porosity ± SD (%)</th>
<th>Mean EE ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>2.89 ± 1.07</td>
<td>84.24 ± 4.54</td>
<td>56.75 ± 17.93</td>
</tr>
<tr>
<td>Polymer content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>2.71 ± 0.65</td>
<td>83 ± 1.94</td>
<td>44.54 ± 10.7</td>
</tr>
<tr>
<td>20</td>
<td>4.75 ± 1.23</td>
<td>74.8 ± 1.55</td>
<td>59.25 ± 2.83</td>
</tr>
<tr>
<td>Organic to aqueous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phase ratio (v/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:1</td>
<td>3.01 ± 0.78</td>
<td>78.07 ± 5.9</td>
<td>47.49 ± 24</td>
</tr>
<tr>
<td>2:1</td>
<td>2.64 ± 0.62</td>
<td>86.42 ± 1.8</td>
<td>62.06 ± 13.42</td>
</tr>
<tr>
<td>Homogenization rate (rpm)</td>
<td>8500</td>
<td>6.24 ± 2.33</td>
<td>74.8 ± 3.23</td>
</tr>
<tr>
<td>22,500</td>
<td>2.24 ± 0.47</td>
<td>84.19 ± 4.32</td>
<td>69.96 ± 10.89</td>
</tr>
<tr>
<td>PDLGA copolymer composition (w/w)</td>
<td>75/25</td>
<td>4.53 ± 1.36</td>
<td>76.25 ± 2.27</td>
</tr>
</tbody>
</table>

* Significant compared to the reference.
** Significant compared to another specimen, which is not the reference.

a The reference sample was prepared using the following conditions: emulsion based on 50/50 PDLGA, 17.5% (w/v) polymer, 2% (w/w) FTS, O:A = 4:1 (v/v), homogenization rate = 16,500 rpm.

3. Results and discussion

Farnesylthiosalicylate (FTS, Salirasib) is a new targeted non-toxic drug which can be used for prevention of restenosis when released from a vascular stent as well as for treatment of cancer, as already mentioned in Section 1. In our composite fibers, the dense core enables obtaining the desired mechanical properties. The drug is located in a porous shell and should therefore not affect the mechanical properties. The shell is highly porous so as to enable release of relatively hydrophobic drugs in a desired manner. In order to characterize our FTS-eluting core/shell fiber platform, we studied FTS release from the fibers, and the shells’ morphology and degradation profiles. Since mechanical properties are important for the stent application, degradation of the tensile properties of the fibers was also studied.

3.1. Drug release and degradation

The freeze drying technique that was used for the shells’ preparation is unique in being able to preserve the temporal state of the emulsion in a solid form. We used this technique in order to produce inverted emulsions in which the continuous phase contained polymer and drug dissolved in a solvent, with water being the dispersed phase. In the current study we investigated the effects of the inverted emulsion’s parameters, i.e. polymer content, drug content, organic:aqueous (O:A) phase ratio and copolymer composition on the shells’ microstructure and on the drug release profile. FTS is a relatively hydrophobic drug. Therefore, most of the emulsion formulations we chose were based on 50/50 PDLGA—the poly(α-hydroxyl acid) with the highest degradation rate in order to be able to release it at an appropriate rate. Based on our experience with paclitaxel-loaded composite fibers, we chose an emulsion formulation containing 12.5% (w/v) 50/50 PDLGA in the organic solution, 2% (w/w) FTS (relative to the polymer load), and an organic to aqueous (O:A) phase ratio of 4:1 (v/v) as the reference sample.

The diameter of the treated core fibers was in the range of 200–250 μm and a shell thickness of 30–70 μm was obtained for most emulsion formulations. The shell’s porous structure contained round-shaped pores in all the specimens that were based on stable emulsions, usually within the 2–7 μm range, with a porosity of 75–92%. The shell microstructure was uniform in each sample, probably due to rapid quenching of the emulsion, which enabled preservation of its microstructure. The pores were partially interconnected by smaller inner pores. The emulsions were stable only within a certain formulation range. The encapsulation efficiency of all studied samples was in the 40–70% range and all specimens contained 33.3 ± 4.8 mg FTS. The structural characteristics of the shell and encapsulation efficiency values of all examined specimens are

![Fig. 1. The effect of copolymer composition on: (a) FTS cumulative release profile from the fibers, (b) degradation profile (expressed as log 10 weight-average molecular weight) of the porous shell, (c) weight loss profile (expressed as w/w) of porous shell. (▲) 75/25 PDLGA, (□) 50/50 PDLGA, (○) 50/50 PDLGA.](image-url)
summarized in Table 1. As mentioned above, FTS is a new drug. We tested its chemical stability using HPLC and found no minor peaks other than the FTS peak nor any changes in elution time even after 100 days in an aqueous medium at 37 °C, indicating that the drug is stable under these conditions.

3.2. Effect of copolymer composition

The effect of the copolymer composition, i.e. the relative quantities of lactic acid (LA) and glycolic acid (GA) in the copolymer, on the drug release profile and on the shell microstructure was found to be the most pronounced of all parameters tested. The FTS release profile from a shell based on 50/50 PDLGA (the reference sample) and from a shell based on 75/25 PDLGA prepared using similar formulation and process parameters are presented in Fig. 1a. Both release profiles exhibited a burst effect accompanied by a release rate which decreased with time. The 50/50 PDLGA sample released 62% of the encapsulated drug during the first day of release, whereas the 75/25 PDLGA sample released only 30%. This difference is attributed mainly to differences in the hydrophilic/hydrophobic nature of these two copolymers. The 50/50 PDLGA copolymer contains more glycolic acid groups and fewer lactic acid groups along the polymer chain and is therefore less hydrophobic than the 75/25 PDLGA and probably exhibits higher water uptake during the initial phase of release. Consequently, this enables more rapid water inflow which results in a higher burst release. Furthermore, the rate of release from the 50/50 PDLGA formulation is slightly higher than the rate obtained with the 75/25 PDLGA formulation and after 2 weeks of degradation the 50/50 formulation released 100% of the drug, whereas the 75/25 formulation released only 79%. This difference is attributed to difference in the degradation rate of these two copolymers, which assists the drug's diffusion. The 50/50 copolymer degrades faster than the 75/25 copolymer and therefore releases the drug at a faster rate. The degradation profiles of the two copolymers are presented in Fig. 1b. The slope of the 50/50 PDLGA's log 10 of the weight-average molecular weight is indeed significantly higher than that of the 75/25 PDLGA. It should be noted that both polymers exhibited an exponential decrease in molecular weight with time. An exponential decay degradation profile of porous PDLGAs was also reported by Wu and Ding (2004) and Lu
The weight loss (erosion) profiles of both polymers exhibited a small weight loss of less than 10% during the first 3 weeks of degradation, whereas after 3 weeks of degradation the 50/50 PDLGA exhibited a fast weight loss while the 75/25 PDLGA did not erode during the measured time period (Fig. 1c), as expected. These results indicate that most of the FTS is released from our porous coatings before they undergo massive weight loss.

The changes of the shell structures of both copolymers during the first 2 weeks of exposure to the aqueous medium are presented in Fig. 2. The starting point (day 0) shows highly porous delicate structures with round pores for both samples. The pore size of the 50/50 PDLGA shell (Fig. 2a) is significantly lower than that of the 75/25 PDLGA (Fig. 2b) and its porosity is higher (Table 1), which enables more surface area for diffusion. After 7 days of degradation in the aqueous medium the 50/50 PDLGA exhibited a rougher structure (Fig. 2c). Most pores disappeared, probably due to water uptake and swelling. At this point of time the 75/25 PDLGA showed only slight changes in morphology compared to the 50/50 PDLGA sample (Fig. 2d), probably due to a lower water uptake. After 14 days of degradation the 50/50 PDLGA exhibited a totally rough porous structure, while at the same time the 75/25 PDLGA began exhibiting a rough structure but the porous features remained (Fig. 2f). It is therefore suggested that structural changes of our highly porous structures resulting from water uptake affect the FTS release profile from the coated fibers.

The shells’ structural changes were further investigated by ESEM using a special technique which enabled us to take consecutive images at different time points at approximately the same locations. Observations of the 50/50 PDLGA samples indicated a decrease of approximately 20% in the film’s thickness during
the first week of exposure to the aqueous medium, followed by an increase in the film’s thickness during the following 2 weeks (Fig. 3a). We hypothesized that although water penetrates the porous structure, it shrinks during the first week due to the change from a porous to a bulk structure and then expands, probably due to swelling. It should be noted that the porous structure gradually converts from a porous to a dense structure and the round pores vanish while the dimensions of the polymer walls increase. The dimensions of polymers usually increase due to swelling. However, most of our structure contains voids rather than polymer. Thus, as the polymer swells, pores vanish, and the overall thickness decreases until reaching a bulk structure and then increases due to further swelling. The water uptake measurements indicate an increase of 40% in the sample’s weight during the first 48 h, after which it stabilizes (Fig. 3b). This further supports our hypothesis that the early structural changes are due to water uptake rather than degradation or erosion. Since the drug diffusion through the fiber’s shell occurs in an aqueous swollen phase, a relatively high water uptake, such as that of our 50/50 PDLGA porous shell, enables faster diffusion rate of the drug molecules.

It can be concluded that a higher glycolic acid content in the copolymer, i.e. a less hydrophobic copolymer, enables more surface area for diffusion and higher FTS release from the fibers due to a combination of early swelling followed by degradation. Higher water uptake affects the microstructure and results in a higher burst release and a higher degradation rate of the host polymer, which assists diffusion. The contribution of the swelling effect is probably more significant in our system.

3.3. Effect of polymer content

The effect of the polymer content of the organic phase on the drug release from the porous shell is presented in Fig. 4a. Burst effects of 75%, 62% and 44% were obtained for 50/50 PDLGA formulations containing low (7.5%, w/v), medium (12.5%, w/v) and high (20%, w/v) polymer contents, respectively. All formulations released most of the encapsulated drug within 15 days. Pore size increased with polymer content while porosity decreased (Fig. 5a and b and Table 1), resulting in less overall surface area for diffusion. As more polymer is incorporated, a more hydrophobic emulsion is obtained, leading to emulsion instability which results in larger aqueous domains that eventually convert into large pores after freeze drying. The decrease in burst effect by increased polymer content may also have resulted due to denser “polymeric walls” that were created between adjacent pores which slows down the diffusion rate. Some specific interactions may also exist between the drug and the host polymer. A relatively high polymer content thus increases the interactions between the polymer and FTS, resulting in a lower diffusion coefficient which results in slower drug release.

3.4. Effect of the O:A phase ratio

The effect of the O:A phase ratio on the FTS release profile from the composite fibers is presented in Fig. 4b. The release profile from samples derived from emulsions with an O:A = 2:1 is very similar to the profile derived from emulsions with an O:A = 4:1. In contradistinction, an increase in the O:A ratio to 8:1 resulted in a decrease in the burst release and prolonged FTS release for 30 days. These phenomena are attributed to changes in the shell morphology. The relatively high O:A phase ratio of 8:1 (or low aqueous phase content) enables lower porosity (Table 1) and larger polymeric domains between pores (Fig. 5c), which create a barrier to the diffusion of drug molecules. An increased O:A phase ratio in our FTS-eluting systems is therefore an effective way for decreasing the burst release and increasing the release period. The porosity of samples derived from emulsions with O:A ratios higher than 4:1 is not high enough to enable effective release of the water-insoluble agents in systems that contain more hydrophobic antiproliferative drugs, such as paclitaxel (Kraitzer et al., 2008).

3.5. Effect of process kinetics

The effects of the emulsion’s homogenization rate (prepared during a 180 s homogenization) on the drug release from the porous shell and on the shell structure are presented in Figs. 4c and 5d, respectively. The homogenization rate had some effect on the FTS release profile due to changes in the shell’s porous structure. A decrease in homogenization rate from 16,500 rpm to 8500 rpm resulted in a significant increase in mean pore size from 2.89 μm to 6.24 μm with a decrease in porosity from 84.24% to 74.8% (Table 1). The low surface area for diffusion of the low homogenization rate specimen enabled some decrease in the burst release.
3.6. Qualitative model of FTS-eluting systems and comparison to paclitaxel-eluting systems

Our results show that the most important parameter in our system affecting release behavior is the copolymer composition. An increase in the glycolic acid content of the PDLGA copolymer resulted in an increase in the burst effect and release rate during the first 2 weeks, mainly due to higher water uptake and changes in microstructure, but also due to a higher degradation rate of the host polymer (Figs. 1–3). Other parameters affecting the FTS release profile are polymer content, O:A phase ratio, surfactants and homogenization rate. However, the effects of these parameters are less prominent in the tested specimens. In these cases the microstructure determines the surface area available for diffusion, which affects the drug release rate. In general, a higher diffusion rate can be achieved when high porosity is combined with a fine structure of lower pore size. The drug content, whose effect was also studied, but not presented, did not exhibit any effect on either the shell microstructure or the release profile.

A qualitative model describing the process → structure → property (drug release profile) effects in our FTS-eluting system can be described as follows (Fig. 6): there are two routes by which the process affects the drug release profile: direct and indirect. The drug release is controlled by diffusion, which may be accelerated by early and late mechanisms:

Direct (process → release profile): the emulsion formulation (especially the host polymer) may affect the water uptake and swelling of the structure and therefore also the FTS burst release. Degra-
Table 2
The tensile mechanical properties of the FTS-eluting fibers.

<table>
<thead>
<tr>
<th></th>
<th>Core polyglyconate fiber</th>
<th>Surface-treated fiber</th>
<th>Core/shell structure*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strength (MPa)</td>
<td>288 ± 32</td>
<td>271 ± 21</td>
<td>268 ± 48</td>
</tr>
<tr>
<td>Young’s modulus (GPa)</td>
<td>1.424 ± 0.085</td>
<td>1.418 ± 0.03</td>
<td>1.353 ± 0.17</td>
</tr>
<tr>
<td>Maximal strain (%)</td>
<td>37 ± 5</td>
<td>32 ± 1</td>
<td>32.7 ± 3</td>
</tr>
</tbody>
</table>

* The effective diameter (of the treated core fiber) was considered.

dation of the host polymer affects the FTS release rate at a later stage.

Indirect (process → structure → release profile): the process effect on the microstructure occurs via an emulsion stability mechanism. The emulsion formulation affects the stability much more than the process parameters (i.e. homogenization rate). The emulsion stability determines the surface area through the microstructure, e.g. the surface area increases when porosity is high and pore size is low. These enables enhanced diffusion rate of the drug from the porous shell and affect both the burst release and later the release.

Diffusion of the drug molecules is thus controlled chemically, mainly by the water uptake and swelling (affected by the emulsion formulation) and also geometrically, through the surface area. The direct effect is more significant than the indirect effect.

It is important to note that most formulations based on 50/50 PDLLA released the FTS molecules within 2 weeks and that the differences between their release profiles resulted mainly from differences in the burst release. Formulations based on host polymers with a higher lactic acid content should be used if FTS release is required for a longer period of time. Furthermore, a higher polymer content and O:A phase ratio or a lower homogenization rate may change the release profile to a lesser extent.

Our experience with paclitaxel-eluting composite fiber structures (Kraitzer et al., 2008) shows that the drug release behavior of this system is different from that of the currently reported FTS-eluting system. Paclitaxel is more hydrophobic than FTS and creates more specific interactions with the host polyesters (Ranade et al., 2004). Therefore, paclitaxel’s diffusion through the host polymer is much slower and massive degradation and erosion of the host polymer must occur in order to enable it. Consequently, changes in formulation parameters affect its release profile mainly after 10 weeks of degradation. In paclitaxel-eluting systems the emulsion formulation influences the diffusion by producing binding regions for the drug as more hydrophobic materials are introduced into the emulsion, thus delaying the drug molecules. The emulsion’s formulation also significantly affects the stability of the emulsion during processing, and as a result determines the freeze dried microstructure. In general, the release profile of FTS from our composite fibers is faster than the paclitaxel release and more adjustable, and therefore more suitable for the stent application. Furthermore, since FTS is less toxic, some burst release may be tolerated.

3.7. Tensile mechanical properties and their degradation

The polyglyconate monofilament sutures were surface-treated, as described in Section 2.2.1, in order to dispose of the fiber’s original coating and enhance the adhesion between the core fiber and the coating. The tensile strength, modulus and ultimate strength of the studied samples are presented in Table 2. It should be noted that in practice, the highly porous shell cannot carry the load. The effective diameter (of the treated core fiber) was therefore used when evaluating the mechanical properties of the core/shell fibers. The tensile strength, Young’s modulus and ultimate strain of the treated and core/shell (coated) fibers are similar to those of the original polyglyconate fibers. There is some decrease in all three tensile properties, probably due to cracks and imperfections caused at the fiber surface as a result of the treatment. However, the changes are not significant (Table 2). This small deterioration in mechanical properties can be avoided in the future if the carrying fiber is extruded specifically for this application instead of using a commercial suture material, evoking the need for surface treatment. Our drug-eluting core/shell fiber structures can therefore be used for biomedical applications which require good initial mechanical properties, such as stents for blood vessel support.

The core/shell fiber samples were immersed in aqueous medium at 37°C in order to evaluate the deterioration of the tensile mechan-
Acknowledgements

The authors are grateful to the Israel Science Foundation (ISF, grant number 1312/07) and to the Slezak Foundation, Tel-Aviv University, for supporting this research.

We would like to thank Concordia Pharmaceuticals (Pt. Lauderdale, FL) for kindly providing us the farnesylthiosalicylate (FTS, Salirasib), Ms. Tami Shpiro and Ms. Tanya Rosenblit, Tel-Aviv University, for their assistance with the drug release studies and Mr. Daniel Giler, Tel-Aviv University, for his assistance with the study of mechanical properties.

References


Halkin, A., Stone, G.W., 2004. Polymer-based paclitaxel-eluting stents in percuta-


The incorporation of this new drug in a stent coating may over-

