Mechanisms of antiproliferative drug release from bioresorbable porous structures

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Abstract: Restenosis (renarrowing of the blood vessel wall) and cancer are two different pathologies that have drawn extensive research attention over the years. Antiproliferative drugs such as paclitaxel inhibit cell proliferation and are therefore effective in the treatment of cancer as well as neointimal hyperplasia, which is known to be the main cause of restenosis. Antiproliferative drugs are highly hydrophobic and their release from porous biodegradable structures is therefore advantageous. The release profiles of four antiproliferative drugs from highly porous polymeric structures were studied in this study in light of the physical properties of both the host polymers and the drug molecules, and a qualitative model was developed. The chemical structure of the polymer chain directly affects the drug release profile through water uptake in the early stages or degradation and erosion in later stages. It also affects the release profile indirectly, through the polymer’s 3D porous structure. However, this effect is minor. The drug volume and molecular area dominantly affect its diffusion rate from the 3D porous structure and the drug’s solubility parameter compared with that of the host polymer has some effect on the drug release profile. This model can also be used to describe release mechanisms of other hydrophobic drugs from porous structures.

Key Words: paclitaxel, farnesylthiosalicilate (FTS), drug-eluting stents, local cancer treatment

INTRODUCTION
Restenosis (renarrowing of the blood vessel wall) and cancer are two different pathologies that have drawn extensive research attention over the years. Antiproliferative drugs such as paclitaxel inhibit cell proliferation and are therefore effective in the treatment of cancer as well as neointimal hyperplasia, which is known to be the main cause of restenosis.

Drug-eluting stents significantly reduce the incidence of in-stent restenosis, which was once considered a major adverse outcome of percutaneous coronary stent implantations. 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.1

Conventional approaches to treating cancer are mainly surgical excision, irradiation, and chemotherapy. In cancer therapy, surgical treatment is usually performed on patients with a resectable carcinoma. An integrated therapeutic approach, such as the addition of a delivery system loaded with an antiproliferative drug at the tumor resection site, is desirable.2,3 This provides a high local concentration of a drug that is detrimental to malignant cells which may have survived surgery, thus preventing tumor regrowth and metastasis.

Four drugs were examined in this study: paclitaxel, sirolimus, farnesylthiosalicilate (FTS, Salirasib), and fluoro FTS (F-FTS). Paclitaxel is the most popular antiproliferative agent. It was originally isolated from a trace compound found in the bark of the Pacific Yew (Taxus brevifolia).4 Its antitumor activity was detected in 1967 by the US National Cancer Institute (NCI) and it was later found to be a promising novel antineoplastic drug. It was approved by the FDA for ovarian cancer in 1992, for advanced breast cancer in 1994 and for early stage breast cancer in 1999. Paclitaxel eventually became a standard medication in oncology.4,5 It acts to inhibit mitosis in dividing cells by binding to microtubules and causes the formation of extremely stable and nonfunctional microtubules. Slow release of perivascularly applied paclitaxel totally inhibits intimal hyperplasia and prevents luminal narrowing following balloon angioplasty. However, paclitaxel’s narrow toxic-therapeutic window may
cause side effects during therapy. Sirolimus, also known as rapamycin, was approved by the FDA in 1999 as Rapamune (Wyeth, NJ), an immunosuppressive drug for transplant rejection. Sirolimus is an immunosuppressive macrolide that easily crosses the cell membrane and binds to an intracellular protein (FKBP12) which activates the mTOR protein. Sirolimus inhibits the cell cycle in the transition from G1 to S, blocking cell proliferation without inducing cell death, thus leading to cell reversion into a quiescent state. It has been found to have potent cell cycle inhibitory activity and therefore inhibits VSMC proliferation.

Farnesylthiosalicylate (FTS, Salirasib) is a new, rather specific, nontoxic drug which was developed at the Tel-Aviv University. It acts as a Ras antagonist, which in its active form (GTP-bound) 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 for active (GTP-bound) Ras and the absence of toxic or adverse side effects were proven in animal models and in humans (Concordia Pharmaceuticals, Ft. Lauderdale, FL). FTS was found to be a potent inhibitor of intimal thickening in the rat carotid artery injury model which serves as a model for restenosis, while it does not interfere with endothelial proliferation. The incorporation of the new drug FTS into a stent coating may overcome the incomplete healing and lack of endothelial coverage associated with current drug-eluting stents. Fluoro FTS is an FTS derivative that contains a fluorine molecule positioned at location 5. This derivative acts to dislodge Ras from cell membranes in a pattern similar to FTS.

The release profiles of paclitaxel and sirolimus have been investigated using various drug-eluting platforms. Drug delivery systems explored so far for localized paclitaxel delivery in cancer treatment include microspheres, surgical pastes, and implants. For example, paclitaxel-loaded PLGA micro and nanofibers (diameters from around 30 nm to 10 μm) were fabricated by electrospinning to treat C6 glioma. Cell viability test results suggested that the paclitaxel-loaded PLGA nanofibers were effective for 72-h incubation. Ranganath et al. developed paclitaxel incorporated poly(ω-lactide-co-glycolide) (PDLGA) implants in the form of microfiber discs and sheets. Paclitaxel was released from the PDLGA copolymer implants (85/15 PDLGA and 50/50 PDLGA) for 80 days. The major limitations of these implants are attaining the required amount of drug for a given amount of time and distributing the antiproliferative drug, such as paclitaxel. The narrow toxic-therapeutic window of these drugs causes side effects and hypersensitivity reactions during therapy. The effectiveness of the drug delivered by polymers depends on whether drug molecules can be transported a sufficient distance from the implantation site. Ranganath et al.’s animal study confirmed brain tumor growth inhibition after 24 days. The stent Taxus contains approximately 50–200 μg (1 μg/mm²) paclitaxel, where ~2 μg are released within 15 days and 92.5% remain in the matrix for a long time. Controlled release of antiproliferative drugs from various polymeric systems for stent applications and local cancer treatment is well described in a review article that has just been published. It is clear from these studies that since antiproliferative agents are water insoluble, high surface area devices should be developed in order to obtain their release in a desired manner.

We have recently developed and studied bioresorbable core/shell fiber structures which release antiproliferative drugs in a controlled manner for stent applications and local cancer treatment. In our composite fibers, the dense core enables obtaining the desired mechanical properties. The drug is located within a porous shell around the core fiber so as not to affect the mechanical properties. The shell is highly porous in order to enable release of the relatively hydrophobic antiproliferative drugs in the desired manner. These fibers can be used for both applications, i.e., for local cancer treatment implanted during tumor resection and as basic elements of the stent drug-eluting coating (shell) can also be applied on metal stents. Our structures are composed of a dense polyglyconate core and a porous drug-loaded PDLGA shell, prepared using freeze drying of inverted emulsions. We examined the effect of the emulsion’s formulation on the release profile of paclitaxel and FTS and found that the host copolymer’s composition exhibited the most profound effect. These systems were further investigated in the current research in order to elucidate the factors that affect the release of antiproliferative drugs. We examined the release profiles of additional drugs (sirolimus and F-FTS) and molecular simulations were performed in order to evaluate the physical properties of the drugs and the host polymers, such as the solubility parameter, three-dimensional structure and molecular area. These enabled us to suggest thorough mechanistic insights for controlled release of antiproliferative drugs from biodegradable structures. In addition to the scientific and medical importance of these new findings, they also enable better understanding of other controlled release systems that release highly hydrophobic drugs for various biomedical applications.

**MATERIALS AND METHODS**

**Materials**

Maxon™ polyglyconate monofilament (3-0) sutures, with a diameter of 0.20–0.25 mm, Syneture, USA, were used as core fibers. This polymer contains a 67.5:22.5 glycolic to trimethylene carbonate ratio.

Bioresorbable porous structures (the shell coating) were made of the following polymers: 75/25 poly(ω-lactide-co-glycolic acid), inherent viscosity (i.v.) = 0.65 dL/g (in CHCl₃ at 30°C, ~97,100 g/mole), Absorbable Polymer Technologies, USA. This polymer is termed 75/25 PDLGA. 50/50 poly(ω-lactide-co-glycolic acid), inherent viscosity (i.v.) = 0.56 dL/g (in CHCl₃ at 30°C, ~31,300 g/mole), Absorbable Polymer Technologies, USA. This polymer is termed 50/50 PDLGA.

The incorporated drugs: Farnesylthiosalicylate (FTS, Salirasib) and fluoro-FTS (F-FTS) were received as a gift from Concordia Pharmaceuticals (Ft. Lauderdale, FL).

- Paclitaxel (Genexol™) was purchased from SamYang Corp. (Seoul, Korea).
aqueous (O:A) phase ratio of 4:1 v/v.

Preparation of core/shell fiber structures

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.

Emulsion formation. A known amount of 50/50 PDLGA or 75/25 PDLGA was dissolved in chloroform to form an organic solution and FTS or paclitaxel 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. The emulsion formulation used in this study contained 17.5% w/v polymer in chloroform, 2% w/w drug (relative to the polymer load), and an organic to aqueous (O:A) phase ratio of 4:1 v/v.

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 precooled (−105°C) freeze dryer (Virtis 101 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 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.

In vitro drug release studies

The drug-loaded composite core/shell fiber structures were immersed in phosphate buffered saline (PBS) at 37°C and pH = 7.4, in triplicates (5 cm long fibers), in order to determine the release kinetics from the drug-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, extracted from the aqueous medium and measured using HPLC. 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.

The drug content of the medium samples was determined using a Jasco High Performance Liquid Chromatograph (HPLC) equipped with a UV 2075 plus detector and a quaternary gradient pump (PU 2089 plus). A reverse phase (RP) column (ACE 5 C18, inner diameter = 4.6 mm, length = 250 mm) equipped with a column guard and kept at room temperature (25°C) was used for the measurements. The HPLC method specifications for each drug are presented in Table I. All measurements were performed at a flow rate of 1 mL/min without gradient and 100 μL samples were injected with an autosampler (AS 2057 Plus). The area of each eluted peak was integrated using the EZstart software version 3.1.7.

Drug extraction procedure

The drug extraction from the medium was performed as follows: the 3 mL PBS/drug medium was completely removed at each time point and placed in a scintillation vial. 3 mL 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 until reaching 4 mL in each test tube. The drug concentration was then estimated using HPLC. An extraction factor was used for correction. Known weights of drug 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 values of the method were 88.4% for FTS, 66.7% for paclitaxel, 84.6% for F-FTS, and 79.5% for sirolimus. The values of the measured drugs were corrected accordingly.

Residual drug recovery from the composite fibers

On the final day of the in vitro study, residual drug from the composite fibers was measured as follows: the fibers were

<table>
<thead>
<tr>
<th>Drug</th>
<th>Method (Mobile Phase)</th>
<th>A (nm)</th>
<th>Injection Volume (μL)</th>
<th>Column</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>45% ACN 55% DDW</td>
<td>227</td>
<td>100</td>
<td>RP Zorbax ODS 5 μm, inner diam. 4.6 mm, length 150 mm</td>
<td>~4.5</td>
</tr>
<tr>
<td>FTS</td>
<td>70% ACN 30% PBS (30 mM, pH = 4.5)</td>
<td>322</td>
<td>100</td>
<td>RP Ace 5 C18 5 μm, inner diam. 4.6 mm, length 250 mm</td>
<td>12–14</td>
</tr>
<tr>
<td>F-FTS</td>
<td>70% ACN 30% PBS (30 mM, pH = 4.5)</td>
<td>322</td>
<td>100</td>
<td>RP Ace 5 C18 5 μm, inner diam. 4.6 mm, length 250 mm</td>
<td>12–14</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>80% ACN 20% PBS (30 mM, pH = 4.5)</td>
<td>224</td>
<td>100</td>
<td>RP Ace 5 C18 5 μm, inner diam. 4.6 mm, length 250 mm</td>
<td>6</td>
</tr>
</tbody>
</table>

○ Sirolimus was purchased from LC Laboratories (MA, USA).

TABLE I. HPLC Method Specifications

- M: pH of mobile phase
- L: Column length (mm)
- m: Inner diameter (mm)
- L: Volume (mL)
- T: Length (mm)
placed in 1 mL methylene chloride for ten minutes 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 reaching 4 mL in each test tube and the drug concentration was estimated by HPLC using the same method as described above. A calibration curve was calculated using known amounts of drug under the same conditions.

The cumulative release profiles were determined relative to the initial amount of drug in the composite fibers (quantity released during the incubation period + the residue remaining in the fibers). All experiments were performed in triplicate. Results are presented as means ± standard deviations.

In vitro degradation and weight loss profiles of the porous PDLGA structures

Porous 50/50 PDLGA or 75/25 PDLGA film structures were fabricated using the emulsion formulation of the reference samples 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), ~1 cm², was incubated in 40 mL PBS containing 0.05% (v/v) sodium azide (as preservative) at 37°C under static conditions for 5 weeks (50/50 PDLGA) or 17 weeks (75/25 PDLGA). PBS was added when the pH was out of range (between 7 and 8) or when the PBS volume dropped below 40 mL. The samples were taken out at weekly intervals, dried using a vacuum oven (35°C for 2 h), and stored in a dessicator. These samples were then used for molecular weight measurements and weight loss measurements.

The weight-average molecular weight of the samples was determined by gel permeation chromatography (GPC). The dried samples of two copolymers were dissolved in methylene chloride before elution to achieve a minimal concentration of about 0.13% w/v. The GPC (Waters 21515 isocratic pump, operating temperature 40°C using a column oven) was equipped with a refractive index detector (Waters 2414, operating temperature 40°C) and calibrated with poly-l-lactic acid MW kit standards (Polysciences, USA). Data were analyzed using the Breeze version 3.3 software. The samples were dissolved in methylene chloride, filtered, and eluted through 4 Styragel columns (model WAT044234 HR1 THF, WAT044237 HR2, WAT044225 HR4, WAT054460 HR5, 300 x 7.8 mm, 5 μm particle diameter) equipped with a guard column at a flow rate of 1 mL/min. The elution medium was Baker analyzed HPLC grade methylene chloride.

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_t) with the initial mass (w_0) as shown in Eq. (1). The results are presented as means ± standard deviations (n = 3).

Water uptake of the porous PDLGA structure

Porous 50/50 and 75/25 PDLGA film structures were fabricated as described above in the paragraph on the in vitro degradation study. Each film sample was cut into 1 × 1 cm² pieces and 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 equation 2 (w is the wet weight at each time point and w_0 is the dry weight measured before the incubation):

\[
\text{Water uptake} = \frac{w - w_0}{w_0} \times 100\% \tag{2}
\]

Solubility parameters and drug-polymer interaction calculations

Calculations of the interaction between the polymer and the drug molecules were carried out using the commercial molecular modeling package “Material Studio” by Accelrys. Two modules were used:

1. Amorphous Cell- A forcefield set of simulation tools used for building, refining and properties calculation of amorphous cells.

2. Discover- A forcefield simulation tool performing molecular mechanics tasks such as minimization of a molecular structure and molecular dynamics tasks. Forcefield dynamics calculations were performed in time steps of 1 fs using the COMPASS forcefield.

Copolymers were formed using the polymer builder function according to the corresponding copolymer ratio in a randomized form. A total of eight computer samples were created using the Amorphous Cell module: four samples of pure material and four samples of the drug/polymer complex. For each sample, 5 cubic cells of about 20 Å edge were built using the experimental density. All the different cells were subjected to 200,000 dynamic steps of 1 fs until reaching equilibrium and then an additional 300,000 steps in order to collect the data, using the Discover module. The cohesion energy of all the samples, solubility parameters of the polymers, the drug molecules and their corresponding interaction distances were calculated using the Discover module Analysis functionality.

RESULTS AND DISCUSSION

Drug release profiles as affected by the physical characteristics of the host polymers

The drug release profiles from shell structures based on 50/50 PDLGA and 75/25 PDLGA are presented in Figure 1 for fibers loaded with paclitaxel and sirolimus [Fig. 1(a)] and for fibers loaded with FTS and F-FTS [Fig. 1(b)]. The water uptake, profile and degradation and weight loss profiles are presented in Figure 2(a–c). Only small amounts of paclitaxel were released from the 50/50 PDLGA and 75/25 PDLGA polymers after two months (Table II), whereas most
of the encapsulated paclitaxel was released from the 50/50 PDLGA shell during week 10–30 [Fig. 1(a)] after intensive degradation and erosion of the host polymer (Fig. 2). In our system the highly hydrophobic paclitaxel is probably attached to the surface of the hydrophobic 75/25 PDLGA even after intensive degradation. It is clear that in spite of the highly porous shell structure which provides a large surface area for diffusion, intensive degradation and erosion of the host polymer is necessary in order to obtain release of the highly hydrophobic paclitaxel. Other investigators working on paclitaxel-eluting systems also reported its relatively slow release rate from various polymeric systems.23,24 Sirolimus was released from the 75/25 PDLGA shell at an even slower release rate. It exhibited a burst release of 40% from the 50/50 PDLGA shell and the rest was released only after intensive degradation and erosion of the host polymer [Figs. 1(a) and 2].

The FTS and F-FTS release profiles from our fiber platform are different from those of paclitaxel and sirolimus. They exhibited a burst effect accompanied by a release rate which decreased with time [Fig. 1(b)]. The 50/50 PDLGA fiber released 86% of the encapsulated FTS and 74% of the encapsulated F-FTS after 1 month of release, whereas the 75/25 PDLGA fiber released only 36 and 37%, respectively. This difference is attributed mainly to differences in the hydrophilic/hydrophobic balance 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 75/25 PDLGA and exhibits higher water uptake during the initial phase of release [Fig. 2(a)]. This enables more rapid water inflow which results in a higher burst release.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cumulative Release (%) from 50/50 PDLGA Matrix After 1 Month</th>
<th>Cumulative Release (%) from 75/25 PDLGA Matrix After 2 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>10.06 ± 0.13</td>
<td>6.05 ± 0.83</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>53.13 ± 7.53</td>
<td>2.66 ± 1.90</td>
</tr>
<tr>
<td>FTS</td>
<td>86.59 ± 6.21</td>
<td>36.34 ± 1.60</td>
</tr>
<tr>
<td>F-FTS</td>
<td>74.71 ± 15.46</td>
<td>37.58 ± 7.42</td>
</tr>
</tbody>
</table>
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. 2(c)], as expected. These results indicate that most of the FTS and F-FTS is released from our porous 50/50 PDLGA coatings before they undergo massive weight loss. When encapsulated in the 75/25 PDLGA, only one third of the encapsulated drug is released after 1 month and a second phase of release in which the rest of the encapsulated drug is released is expected to occur only after intensive degradation of the host polymer.

In our previous research we showed that the process effect on the microstructure occurs via an emulsion stability mechanism. The emulsion stability determines the surface area through the microstructure. Since the emulsion stability of the 50/50 PDLGA is higher than that of the 75/25 PDLGA, the former exhibits a finer structure with smaller pores, which results in larger porosity and a larger surface area for diffusion (Fig. 3). This enables an enhanced diffusion rate of the drug from the porous shell of the 50/50 PDLGA compared to the 75/25 PDLGA and affects both the burst release and later the release. The differences between the release rates of FTS and F-FTS from 50/50 PDLGA and 75/25 PDLGA are therefore attributed mainly to water uptake of the host polymers and structural differences. The degradation rate affects the release rate at a more advanced stage.

It is also important to note that based on our experience with highly hydrophobic drugs encapsulated in PDLGAs, we assumed that part of the encapsulated drug is in solid solution with the polymer and part of it (above solubility) stays in small clusters in the polymer. Since the drug was originally spread in the organic phase of the inverted emulsion and not in the aqueous one, all drug clusters are probably located in the polymer and not on the surfaces of the pores. Thus, the diffusion of the drug through the polymeric structure is affected by the shell’s microstructure. The porosity values of 50/50 PDLGA and 75/25 PDLGA are very similar (67 and 69%, respectively). Thus, the smaller pore size of the 50/50 PDLGA, compared with that of the 75/25 PDLGA, contributes to the faster release of paclitaxel from the former.

In conclusion, our drug delivery, water uptake, and degradation results clearly show that although antiproliferative drugs are highly hydrophobic, their release profiles from biodegradable polymers can be totally different. Some drugs, such as paclitaxel and sirolimus, can be totally released only after intensive degradation of the host polymer, and other drugs, such as FTS and F-FTS, can be partially released even as a result of some water uptake, a short time after being immersed in an aqueous medium. To further investigate these systems, molecular simulations were performed so as to evaluate the physical properties, such as the solubility parameter, chemical structure, and molecular area of the investigated drugs and host polymers. These are presented in the following section.

The physical properties of the drugs and polymers and their effect on the drug release profile

**Solubility parameter.** The Hildebrand solubility parameter, \( \delta^{25} \), is defined as the square root of the cohesive energy density. It is important to note that materials with similar \( \delta \) values are likely to be miscible. The solubility parameters of the four drugs (paclitaxel, FTS, sirolimus, and F-FTS), the two polymers (50/50 PDLGA and 75/25 PDLGA) and the various polymer–drug complexes were evaluated using the Discover simulation software. The results are presented in Table III. The solubility parameters of paclitaxel and FTS (21.15 (J/cm\(^3\))\(^{1/2}\) and 21.06 (J/cm\(^3\))\(^{1/2}\) respectively) are almost the same and are very similar to that of 75/25 PDLGA (21.34 (J/cm\(^3\))\(^{1/2}\)) and lower than that of 50/50 PDLGA (23.91(J/cm\(^3\))\(^{1/2}\)). This means that both drugs are slightly more soluble in 75/25 PDLGA than in 50/50 PDLGA and explains the slower diffusion rate from the 75/25 PDLGA during early stages of release, before intensive degradation occurs (Figs. 1 and 2). The solubility parameter of sirolimus [18.63 (J/cm\(^3\))\(^{1/2}\)] is much lower than that of 50/50 PDLGA and it therefore diffuses out more easily from this polymer compared with paclitaxel.

The solubility parameters of the polymer–paclitaxel and polymer–FTS complexes were lower than those of the pure polymers (Table III), indicating a nonspecific interaction between these drugs and the polymers. Since the solubility
parameters of FTS and paclitaxel are very similar to those of 75/25 PDLGA, we can assume that there are probably some hydrophobic interactions between the drugs and the host polymer, rather than specific strong interactions. The solubility parameters of the drug/polymer complexes obtained for sirolimus and F-FTS were similar to those of the pure polymers, indicating possible specific interactions between these drugs and the host polymers.

Chemical structure and surface area. The chemical formulations of the four drugs and their 3D images are presented in Figure 4. The molecular weight, calculated van der Waals volume, and molecular areas of these drugs are presented in Table IV. It should be noted that paclitaxel and sirolimus exhibit relatively high volumes (754 and 842 \( \text{m}^3 \)), respectively) and high molecular areas (744 and 842 \( \text{m}^2 \)), while FTS and F-FTS exhibit much lower volumes (365 and 362 \( \text{m}^3 \)), respectively) and lower molecular areas (415 and 411 \( \text{m}^2 \)). Paclitaxel and sirolimus are approximately twice the size of FTS and F-FTS. Furthermore, paclitaxel and sirolimus have spherical and complex molecular shapes while FTS and F-FTS exhibit a simpler straight linear shape (Fig. 4). The complex shape and large size of paclitaxel and sirolimus probably substantially reduce the diffusion coefficient of the drug molecules, since they lower the molecular mobility of the drug and thus delay its release.

Combined effect of host polymer characteristics and drug physical properties on the release profile—A qualitative model

This research clearly shows that in spite of the highly porous shell structure, the physical properties of both components, polymer and drug, strongly affect the drug release profile of the hydrophobic drugs, in various aspects.
First, the host polymer exhibits both direct and indirect effects on the release profile. The chain chemical structure of the host polymer affects its water uptake and therefore also the burst release of the drug in the early stages. It also affects the release profile in later stages, which occur due to degradation and erosion of the host polymer. It was found that 50/50 PDLGA, which is less hydrophobic than 75/25 PDLGA, exhibits faster water uptake kinetics during the 5 first days of immersion in the aqueous medium and also degrades faster (Fig. 2). Therefore, small narrow drugs such as paclitaxel and sirolimus exhibit faster release rates from 50/50 PDLGA, mainly due to faster water uptake, and bulky complex drugs such as paclitaxel and sirolimus exhibit faster release rates from 50/50 PDLGA, mainly due to a faster degradation rate of the host polymer during advanced stages.

There exists an indirect route as well, which affects the drug release profile through emulsion stability and the resulting porous structure and surface area for diffusion. The surface area increases when the porosity is high and pore size is small. For example, 50/50 PDLGA exhibits finer microstructure than 75/25 PDLGA (Fig. 3), and this affects both the burst release and the later release. This can also explain differences between release profiles from 50/50 PDLGA compared to the release profiles from 75/25 PDLGA of all examined drugs.

The drug characteristics also play a very important role in controlling the release profile from our porous systems. In this study we have shown that drug volume and molecular area strongly affect the drug’s diffusion from the porous shell. For example, small narrow drug molecules such as FTS and F-FTS can diffuse out of the porous structure when some water uptake occurs, while bulky drug molecules such as paclitaxel and sirolimus need intensive degradation of the host polymer in order to diffuse out. This phenomenon can be attributed to the fact that the hydrophobic drug molecules are probably located in the polymeric domains of the porous structure rather than in the pores. Otherwise they would be released much faster from the partially connected porous structure.

Similarity in solubility parameters of the drug and the host polymer also has some effect on the drug release profile. For example, in our system the solubility parameters of paclitaxel and FTS are very similar to that of 75/25 PDLGA and lower than that of 50/50 PDLGA. These drugs thus tend to diffuse out faster from 50/50 PDLGA than from 75/25 PDLGA, due to some solubility effects. However, the “solubility parameter” effect is less important than the “size and shape” effect, otherwise paclitaxel and FTS would show very similar release profiles (due to very similar solubility parameters).

Finally, specific interactions between drug and polymer probably do not affect the drug release profile. According to our simulations, there are probably no specific interactions between paclitaxel or FTS and the host polymers, but there may be specific interactions between sirolimus and the host polymers. Specific interactions between drug and polymer, such as similar solubility, tend to slow the drug release rate. Our evaluations show that the solubility parameter of sirolimus is much lower than that of 50/50 PDLGA, which explains why sirolimus diffuses out of 50/50 PDLGA faster than paclitaxel, in spite of the specific interactions. Such interactions probably play less important role than the “solubility effect”.

In conclusion, highly porous structures for controlled release of hydrophobic drugs such as antiproliferative agents were developed and studied. The effects of both the polymer and the drug structure and physical properties on the drug release profile were studied through a combination of in vitro results and molecular simulations. We conclude that both drug and polymer chain structures strongly affect the release profile of hydrophobic drugs from relatively hydrophobic host polymers. The chemical structure of the polymer chain directly affects the drug release profile through water uptake in the early stages or degradation and erosion in later stages. It also affects the release profile indirectly through the polymer’s 3D porous structure. However, this effect is minor. The hydrophobic drug molecules are probably located in the polymeric domains of the 3D structure, rather than in the pores. The drug volume and molecular area have a dominant effect on the drug’s diffusion rate from the 3D polymeric porous structure. The drug solubility parameter (compared with that of the host polymer) also has some effect on the drug’s diffusion, while specific polymer–drug interactions are probably less important. A schematic representation of this physical model is presented in Figure 5.
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