Antibiotic-eluting bioresorbable composite fibers for wound healing applications: Microstructure, drug delivery and mechanical properties

Jonathan J. Elsner, Meital Zilberman *

Department of Biomedical Engineering, Faculty of Engineering, Tel-Aviv University, Tel-Aviv 69978, Israel

Received 19 October 2008; received in revised form 26 March 2009; accepted 7 April 2009

Available online 21 April 2009

Abstract

Novel antibiotic-eluting composite fibers designed for use as basic wound dressing elements were developed and studied. These structures were composed of a polyglyconate core and a porous poly(DL-lactic-co-glycolic acid) shell loaded with one of three antibiotic drugs: mafenide acetate, gentamicin sulphate and ceftazidime pentahydrate. The shell was prepared by the freeze-drying of inverted emulsions. The fiber investigation focused on the effects of the emulsion’s formulation on the shell microstructure and on the resulting profile of drug release from the fibers. Albumin was found to be the most effective surfactant for stabilizing the inverted emulsions and also to have a beneficial holdup effect on the release kinetics of the hydrophilic antibiotic drugs, especially mafenide acetate, probably through a specific interaction. An increase in the organic:aqueous phase ratio, polymer content or molecular weight of the host polymer resulted in a decrease in the burst release and a more moderate release profile due to changes in shell microstructure. The first two parameters were found to be more effective than the third. The diverse release profiles obtained in the current study and the good mechanical properties indicate that our new composite fibers have good potential for use in wound healing applications.

Keywords: Wound dressings; Fiber; Antibiotic; Poly-(DL-lactic-co-glycolic acid); Controlled drug delivery

1. Introduction

Wound dressings aim to restore the milieu required for skin regeneration and to protect the wound from environmental threats and penetration of bacteria. Although traditional gauze dressings offer some protection against bacteria, this protection is lost when the outer surface of the dressing becomes moistened by wound exudate or external fluids. Furthermore, traditional gauze dressings do not greatly restrict moisture evaporation and may cause dehydration of the wound bed. This can lead to adhesion of the dressing, particularly as wound fluid production diminishes, causing pain and discomfort to the patient when removed [1]. Most modern dressings are designed according to the well-accepted bilayer structure concept, i.e. an upper dense “skin” layer to prevent bacterial penetration and a lower spongy layer designed to adsorb wound exudates and accommodate newly formed tissue [2,3]. Unfortunately, dressing material which has absorbed wound discharge provides conditions that are also favorable for bacterial growth. This has encouraged the development of a new generation of wound dressings with improved curative attributes that provide an antimicrobial effect by eluting various germicidal compounds. These dressings still require frequent changing, which may be painful to the patient, harm the vulnerable underlying skin and increase the risk of secondary contamination. Biodegradable dressings successfully address this shortcoming, since they do not need to be removed from the wound surface once they have fulfilled their role. Film dressings made of lactide–caprolactone copolymers such as Topkin® (Biomet, Europe) and Oprafo® (Lohmann & Rauscher, Germany) are currently available [4]. Biodegradation of
These films occur via hydrolysis of the copolymer into lactic acid and 6-hydroxycaproic acid. However, film dressings are better suited for small wounds, since they lack absorbance and are impermeable to water vapor and gases, both of which cause accumulation of wound fluids on larger wound surfaces.

Some recently reported work has focused on an alternative on the development of more complex biodegradable fiber-based wound dressings with antibiotic delivery [5–8]. Fiber-based dressings composed of either continuous fibers that form a non-woven fiber mesh or a fabric made from woven fibers offer a high surface area for controlled release, absorbency and pliability. However, the main challenge in designing a device for the release of low molecular weight (MW) hydrophilic antibiotics is to overcome the rapid discharge of the drug from the device. This drawback has also been reported for other antibiotic-eluting devices such as periodontal devices [9,10] and vascular grafts [11,12]. The local antibiotic release profile should exhibit a considerable initial release rate in order to respond to the elevated risk of infection from bacteria introduced during the initial trauma, followed by a release of antibiotics at an effective level long enough to inhibit latent infection [13]. The location, size and degree of injury, as well as the rate of tissue regeneration (depending on the patient’s age and other parameters), affect the wound healing process. Hence, characteristic healing has been reported to take 3–7 weeks [14]. Common strategies that have been described in an attempt to overcome the problem of rapid drug release include the entrapment of the hydrophilic drug within a hydrophobic substance as a means to delay water penetration and outward drug diffusion [15,16], or enhancement of drug bonding to the carrying matrix [11,12,17]. The latter can be achieved either by selecting or modifying a matrix material to support the formation of covalent bonds. Van der Waals dispersion forces, hydrogen bonds, or ionic interactions between the drug and the matrix. Vascular grafts sealed with albumin and gelatin have been shown to promote such interactions and therefore demonstrate a reduced burst release of antibiotics compared to uncoated grafts [11,12]. A wound dressing based on succinylated collagen, which behaves as an anion after swelling, has been shown to delay the release of the cationic drug ciprofloxacin via ionic interactions [17].

Incorporation of antibiotics in the process of fiber spinning (e.g. electrospinning or melt and solution spinning) is associated with the disadvantages of poor mechanical properties due to drug incorporation and limitations in drug loading. Furthermore, many drugs and proteins do not tolerate melt processing and organic solvents. The main goal of the current study was therefore to develop and study new fiber structures loaded with antibiotics. Our composite fibers combine a dense polymer core fiber and a drug-loaded porous shell structure, i.e. the drug is located in a separate compartment (a “shell”) around a melt spun “core” fiber. The shell is prepared using freeze-drying of inverted emulsions. This fabrication process uses mild processing conditions and is designed to produce a structure with good mechanical properties as well as the desired drug-release profile. These new fibers are ideal for forming thin, delicate, biomedically important structures such as wound dressings. Our fibers were loaded with one of three antibiotic drugs: gentamicin sulphate, ceftazidine pentahydrate and mafenide acetate. The first two antibacterial drugs are broad-spectrum antibiotics which can be used systemically or locally, whereas the third is typically used in burn dressings. The drugs’ physicochemical properties and antibacterial spectra are presented in Table 1. The effects of the emulsion’s formulation parameters on the shell microstructure and on the resulting drug-release profile and mechanical tensile properties are presented.

2. Materials and methods

2.1. Materials

Maxon™ polyglyconate monofilament sutures with a diameter of 0.20–0.25 mm (United States Surgical Inc., USA) were used as core fibers.

Bioresorbable porous structures (the shell coating) were made of 75/25 poly(DL-lactic-co-glycolic acid) (PDLGA), inherent viscosity (i.v.) = 0.4, 0.65 and 1.13 dL g⁻¹ (in CHCl₃ at 30 °C), MW approximately 50, 100, 240 kDa, respectively (Absorbable Polymer Technologies, Inc., USA).

2.1.1. Drugs

Gentamicin sulfate (cell-culture tested), 590 µg gentamicin base per mg of salt (Sigma, G-1264).

4-Aminomethylbenzenesulfonamide acetate salt (mafenide acetate) (Sigma, A-3305).

Ceftazidime hydrate, 90–105% (Sigma, C-3809).

2.1.2. Surface active agents

Bovine serum albumin (BSA), MW = 66,000 Da (Sigma, A-4503).

Poly(vinyl alcohol) (PVA), 87–89% hydrolyzed, MW = 13,000–23,000 Da (Aldrich, 36,317-0).

2.1.3. Reagents

Isopropyl alcohol (propanol) was purchased from Frutarom, Israel.

1,1,1,3,3,3-hexafluoro-2-propanol (H1008) was purchased from Spectrum Chemical Mfg. Corp.

2.2. Preparation of core–shell fiber structures

2.2.1. Fiber surface treatment

The sutures were surface-treated in order to dispose of the original fiber coating and to enhance adhesion between the core fiber and the coating. The Maxon™ fibers were gently wrapped around flexible Teflon frames and dipped in a Petri dish containing 1,1,1,3,3,3-hexafluoro-2-propanol for 40 s. The fibers were then washed with 70% ethanol and dried.
2.2.2. Emulsion formation

A known amount of PDLGA was dissolved in chloroform to form an organic solution. A known amount of the drug was dissolved in double-distilled water and then poured into the organic phase (in a test tube). Homogenization of this emulsion was performed using a Kinematica PT-3100 Polytron homogenizer operating at 16,000 rpm (medium rate which was found to be optimal) for 2 min. An emulsion formulation containing 15% w/v PDLGA (100 kDa) polymer in the organic solution, 5% w/w drug in the aqueous medium (relative to the polymer load), and an organic to aqueous (O:A) phase ratio of 6:1 v/v was used as the reference formulation. Additional formulations included variations in polymer content (17.5% and 20%), drug loading (1% and 2.5%), O:A phase ratios (2:1, 8:1, 10:1 and 12:1) and polymer MW (50 and 240 kDa). The effect of BSA and PVA as surfactants (0.5% and 1% w/v in the aqueous phase) was also studied. These surfactants are well known as stabilizers for emulsions [18,19]. In each studied series, only one parameter was changed and the others remained the same as in the reference formulation.

2.2.3. Core–shell fiber structure formation

The treated Maxon® core 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 (Virtis 101 equipped with a nitrogen trap) capable of working with organic solvents and freeze-dried in order to preserve the microstructure of the emulsion-based core–shell fiber structures. Drying was performed in two stages:

(i) The freeze-dryer chamber pressure was reduced to 100 mTorr while the temperature remained at −105 °C.
(ii) The condenser was turned off after 5 h and its plate temperature gradually increased to room temperature while the pressure was monitored between 100 and 700 mTorr. During this step the liquid nitrogen trap condensed the excess water and solvent vapors. The samples were stored in desiccators until use.

2.3. In vitro drug-release studies

The composite core–shell fiber structures (triplicate samples, 15 cm each) were immersed in phosphate-buffered saline (PBS, pH 7.0) at 37 °C for 60 days in order to determine the various drug-release kinetics from these structures. The release studies were conducted in closed glass vessels containing 1.5 ml PBS medium. The medium was removed (completely) periodically, at each sampling time (6 h, 1, 2, 3, 7, 14, 21, 28, 35, 42, 49 and 56 days), and fresh medium was introduced.

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Table 1

<table>
<thead>
<tr>
<th>Antibiotic agent</th>
<th>Molecular weight (g mol⁻¹)</th>
<th>Water solubility (mg ml⁻¹)</th>
<th>Serum protein binding*</th>
<th>Antibacterial spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin sulphate</td>
<td>477.6</td>
<td>100</td>
<td>0–30% [31]</td>
<td>Effective against a broad spectrum of Gram-positive and Gram-negative bacteria</td>
</tr>
<tr>
<td>Ceftazidime pentahydrate</td>
<td>546.6</td>
<td>5</td>
<td>0–10% [31]</td>
<td>A third-generation cephalosporin which displays a broad spectrum activity against Gram-positive and Gram-negative bacteria. Unlike most third-generation agents, it is active against Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Mafenide acetate</td>
<td>246.3</td>
<td>250</td>
<td>20–90% [29]</td>
<td>Bacteriostatic for many Gram-negative and Gram-positive organisms, including Pseudomonas aeruginosa and certain strains of anaerobes. Mafenide is highly soluble and diffuses into and through eschar producing a marked reduction in the number of bacteria present, even in avascular tissue of second- and third-degree burns</td>
</tr>
</tbody>
</table>

* Albumin binds drugs at varying degrees, depending on the specific drug’s affinity. The bound portion may act as a reservoir or depot from which the drug is slowly released as the unbound form.
2.3.1. Gentamicin assay

Determination of the medium’s gentamicin content was carried out using an Abbott Therapeutic Drug Monitoring System (TDX, Abbott Laboratories) according to the manufacturer’s instructions. This machine enables the determination of the gentamicin concentration based on a polarization fluoroimmunoassay using fluorescein as a tracer. Briefly, the latter is excited by polarized light. Polarization of the emitted light is dependent on molecule size. Free and labeled drug compete for binding sites. The drug concentration in the sample is proportional to the scatter of polarized light caused by free labeled drug. The measurable concentration range without dilution is 0.0–10.0 μg ml⁻¹. Higher drug concentrations were measured after carrying out manual dilution.

2.3.2. Mafenide assay

The medium’s mafenide content was determined using a Jasco high performance liquid chromatography (HPLC) system with a UV 2075 plus detector and a reverse-phase column (Interstil® ODS-3 V 5 μm, inner diameter d = 4.6 mm, length = 250 mm), kept at 25 °C. The mobile phase consisted of a mixture of PBS and acetonitrile at a flow rate of 1 ml min⁻¹ with a quaternary gradient pump (PU 2089 plus), gradient t = 0 min, 100/0 (v/v), t = 1.5 min, 90/10 (v/v), t = 4 min, 100/0 (v/v). Thirty microliters of samples were injected with an autosampler (AS 2057 Plus). The column effluent was eluted for 9 min and detected at 267 nm. The area of each eluted peak was integrated using EZstart software, v. 3.1.7. A calibration curve was prepared for concentrations ranging from 1.0 to 200.0 μg ml⁻¹ (n = 8, correlation coefficient >0.999, slope 0.0002295).

2.3.3. Ceftazidime assay

The medium’s ceftazidime content was determined using a Jasco high performance reverse-phase column (ODS-3 V 5 μm, inner diameter d = 4.6 mm, length = 250 mm), kept at 25 °C. The mobile phase consisted of a mixture of PBS and acetonitrile (95/5, v/v) at a flow rate of 1 ml min⁻¹ with a quaternary gradient pump (PU 2089 plus), gradient t = 0 min, 100/0 (v/v), t = 1.5 min, 90/10 (v/v), t = 4 min, 100/0 (v/v). Thirty microliters of samples were injected with an autosampler (AS 2057 Plus). The column effluent was eluted for 9 min and detected at 267 nm. The area of each eluted peak was integrated using EZstart software, v. 3.1.7. A calibration curve was prepared for concentrations ranging from 1.0 to 200.0 μg ml⁻¹ (n = 8, correlation coefficient >0.999, slope 0.0000318).

2.4. Residual drug recovery from composite fibers

Residual drug recovery from the composite fibers was measured as follows: the fibers were placed in 1 ml methylene chloride in order to dissolve the remaining PDGLA coating. Two milliliters of water were then added in order to dissolve the hydrophilic drug residues. The materials were vortexed for 30 s and then left to stand until phase separation occurred. The aqueous phase was filtered in order to dispose of polymer particles. The drug concentration was estimated using the above-described assays. The experiments were performed in triplicate.

2.5. Morphological characterization

The morphology of the composite core–shell fiber structures (cross-section of cryogenically fractured fibers) was observed by scanning electron microscopy (SEM) using a JEOL JSM-6300 at an accelerating voltage of 5 kV. The SEM samples were Au sputtered prior to observation. The mean pore diameter (n = 80 pores) and porosity of the observed morphologies for each SEM fractograph were analyzed using Sigma Scan Pro software. Porosity was determined as the area occupied by the pores divided by the total area.

2.6. Microbiological evaluation

Agar diffusion tests (Kirby–Bauer method) were conducted to confirm the bioactivity of the antibiotics encapsulated in the composite fibers. Small discs (n = 3, d = 10 mm) were cut from plain-woven Maxon fibers coated with the reference formulation. The discs were then placed dry on Muller–Hinton agar plates, inoculated with relevant bacteria (one strain on each plate): Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa. Bacterial inhibition zones around the dressings were assessed after 24 h of incubation. Similar dressings, prepared without drug, were used as controls.

2.7. Tensile mechanical properties

The fibers’ tensile mechanical properties were measured at room temperature, under unidirectional tension at a rate of 50 mm min⁻¹ (ASTM D 3379), using a 5500 Instron machine. 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. Six samples were tested for each type of specimen.

2.8. Statistics

Statistics were calculated using SPSS 10 software. All data are expressed as means ± standard deviation (SD). ANOVA (Tukey–Kramer) was used for group comparison and P values less than 0.05 were considered significant.

3. Results

3.1. Morphological characterization

Composite fiber structures with a core diameter of approximately 280 μm and a shell thickness of 20–50 μm were produced and studied. A SEM fractograph showing...
Fig. 1. SEM fractographs of a mafenide-loaded composite fiber (reference formulation) demonstrating the concept of core–shell fiber structures.

Fig. 2. Shell microstructure of samples containing the reference formulation (5% w/w drug, 15% w/v polymer and O:A phase ratio of 6:1). Loaded drug: (a) mafenide, (b) mafenide + 1% w/v albumin, (c) ceftazidime, (d) ceftazidime + 1% w/v albumin, (e) gentamicin, (f) gentamicin + 1% w/v albumin.
the bulk morphology of the reference specimen formulation containing mafenide is presented in Fig. 1a. The quality of the interface between the fiber and the porous coating is high (Fig. 1b), i.e. the surface treatment enables good adhesion between the core and the shell. The shell’s porous structure contains round pores with a diameter of 0.5–5 μm. The shell’s microstructure is relatively uniform regardless of the type of antibiotic incorporated within the shell (Fig. 2). The morphology of the shell structures loaded with mafenide, ceftazidime and gentamicin are presented in Fig. 2a–f, respectively. However, the incorporation of the various antibiotics in the aqueous phase resulted in slight changes in the shells’ characteristic morphology (Fig. 2a, c and e). This is attributed to differences in the interfacial tension between the organic and aqueous phases for the different drugs, with a mean pore diameter of 1.5 μm for ceftazidime, 2 μm for mafenide and 4.8 μm for gentamicin-loaded fibers. BSA demonstrated the best stabilizing effect on the emulsion, which was reflected by a finer microstructure and a moderation of the above-described microstructural differences. Pore size was reduced to values of 1, 1.1 and 1.3 μm, respectively (Fig. 2b, d and f). Further investigation of the shell’s structural characteristics focused on the effects of four emulsion composition parameters: O:A phase ratio, drug concentration in the aqueous phase, polymer content in the organic phase and polymer MW (i.v.) derived from emulsions not containing a surfactant. The results for ceftazidime-loaded fibers are summarized in Table 2.

The modification of the emulsion’s O:A phase ratio predominantly affected the porosity of the shell structure, but not the pore size (Table 2). Low O:A ratios are associated with a higher volume fraction of pores (89% for O:A 2:1) and connectivity between pores, whereas higher O:A ratios (12:1) are associated with thicker polymer walls (between pores) and a larger portion of pores that become isolated (Fig. 3b). The drug concentration in the aqueous phase did not exhibit a considerable effect on the shell’s structure, although it was generally limited by the 10% w/w fraction above which destabilization of the emulsion occurred resulting in a non-homogeneous shell. An increase in the polymer content and the employment of higher MW

<table>
<thead>
<tr>
<th>Process parameter</th>
<th>Value</th>
<th>Pore diameter (μm)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime content (% w/w)</td>
<td>1</td>
<td>2.5 ± 0.8</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1.7 ± 0.6</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.5 ± 0.6</td>
<td>68</td>
</tr>
<tr>
<td>Organic to aqueous phase ratio (v/v)</td>
<td>2</td>
<td>N.A</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.5 ± 0.6</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.2 ± 0.5</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.8 ± 0.4</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.6 ± 0.4</td>
<td>45</td>
</tr>
<tr>
<td>Polymer content (% w/v)</td>
<td>12.5</td>
<td>1.5 ± 0.8</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>1.5 ± 0.6</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>1.0 ± 0.5</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>1.2 ± 0.9</td>
<td>22</td>
</tr>
<tr>
<td>Approximate polymer MW (kDa)</td>
<td>50</td>
<td>1.3 ± 0.4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.5 ± 0.6</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>0.5 ± 0.4</td>
<td>16</td>
</tr>
</tbody>
</table>

Fig. 3. SEM fractographs of core–shell fibers showing the effect of change in certain formulation parameters on shell microstructure. (a) Reference formulation 5% w/w ceftazidime, 15% w/v polymer (75/25 PDLGA, MW 100 kDa), O:A phase ratio of 6:1, (b) O:A modified to 12:1, (c) polymer content modified to 20% w/v polymer, (d) higher polymer MW (modified to 240 kDa).
polymers (until the verge of instability—to be discussed later) resulted in a similar trend of decrease in pore size (Fig. 3c and d, respectively), since high viscosities associated with the increase in these two parameters help reduce flocculation and coalescence of droplets by reducing the tendency of droplets to move. Similar trends were observed also for gentamicin- and mafenide-loaded samples.

3.2. In vitro drug-release studies

Typical total drug loadings for fibers based on the reference formulation for the three studied drugs were similar: gentamicin 9.1 ± 0.4 µg cm⁻¹, ceftazidime 8.1 ± 0.4 µg cm⁻¹ and mafenide 8.1 ± 0.2 µg cm⁻¹. In vitro drug-release profiles from these (reference) fibers present a burst release of 85–95% after 6 h, with almost total release of the drug within a week. There is practically no difference in the release kinetics of the three drugs. Nonetheless, a range of different release profiles which vary in their initial burst release and total duration of release were obtained with variations of the reference formulation.

Of the three studied antibiotics, the release of mafenide was most affected by the incorporation of surfactants. We therefore chose to present the effects of surfactants on composite fibers loaded with mafenide (Fig. 4). The incorporation of 1% albumin in the reference formulation resulted in the lowest burst release (53% compared to 72% with 1% PVA, and 85% without surfactant) and yielded controlled release of the drug over a period of 50 days. The effect of albumin as a surfactant on reference fibers loaded with the three antibiotic drugs is presented in Fig. 5b. It can be seen that refinement of the microstructure by stabilization with albumin helps reduce the burst release to some degree for gentamicin and ceftazidime, and more notably for mafenide, probably due to specific interactions with albumin. Other modifications of the formulation were made to promote better restraint of outward drug diffusion. These included an increase in the O:A phase ratio, polymer concentration and polymer MW. The effect of each of these formulation parameters was studied individually without adding a surfactant, and is presented in Fig. 6. An increase in the O:A phase ratio from 6:1 to 12:1 reduced the burst release of ceftazidime from 95% to 44%, although total release of the encapsulated drug still occurred within 1 week. The effect of an increase in
polymer content in the organic phase from 15% to 20% w/v, or an increase in the polymers’ MW from 100 to 240 kDa, resulted in a notable decrease in the drug’s diffusion rate out of the shell. Typical burst release in both cases was around 30%, and drug release from these fibers lasted 40-50 days. In conclusion, an increase in the O:A phase ratio or a decrease in polymer content or MW of the host polymer enabled a lower burst release and a more moderate release profile.

3.3. Microbiological results

Newly fabricated (dry) drug-loaded samples placed on cultures of the three relevant bacterial species displayed clear inhibition zones around them as shown, for example, in Fig. 7 (left) for ceftazidime-loaded samples. Conversely, control samples coated with the same formulation without the drug, did not demonstrate any bactericidal effect (Fig. 7, right). All three bacterial strains demonstrated susceptibility to the antibiotics used in the study.

3.4. Tensile mechanical properties

The polyglyconate monofilament sutures were surfacetreated, as described in Materials and methods, in order to dispose of the fiber’s original coating and enhance the adhesion between the core fiber and the coating. The tensile stress–strain curve of the treated fibers closely follows that of the original fiber (Fig. 8), whereas the treated fibers fail at a lower stress (503 MPa) compared to the original fibers (771 MPa), probably due to cracks and imperfections caused at the fibers’ surface as a result of the treatment, which lead to earlier failure.

Two methods were used to evaluate the mechanical properties of the core–shell fibers: (i) using the total diameter of the fiber; and (ii) using an effective diameter which is actually the treated core fiber. The tensile stress–strain curves of fibers coated with the reference emulsion according to both methods are presented in Fig. 8, and the tensile strength, modulus and ultimate strength values for all studied samples are presented in Table 3. The treated core fiber lost 41% of its strength and 51% of Young’s modulus due to its coating. However, it should be noted that in practice, the highly porous shell cannot carry the load. The second method of evaluation therefore affords the real effect of coating, which is a 3% decrease in tensile strength and a 22% decrease in Young’s modulus (Table 3).

4. Discussion

The incorporation of broad-spectrum antibiotics such as gentamicin, ceftazidime and mafenide in wound dressings can help reduce the bio-burden in the wound bed and thus prevent infection and accelerate wound healing. Mafenide is particularly appropriate in cases of burn wounds, since it exhibits excellent antimicrobial activity and the best eschar penetration of any antibacterial agent [20]. However, mafenide causes severe side-effects, especially when applied to large areas [21]. The controlled release of this antibiotic, alone or in combination with the other antibiotics reported in this study, may help prevent the occurrence of complications associated with conventional topical treatment. The controlled release of antibiotics from wound dressings is challenging, since other design considerations need to be addressed. Porosity is desired in order to provide adequate gaseous exchange and absorption of wound exudates [22]. However, it acts as a two-edged
sword since rapid water penetration typically leads to a rapid release of the active agent within several hours to several days.

The process of freeze-drying is unique in its ability to preserve the liquid structure in solids. The microstructure of the freeze-dried inverted emulsion, our fibers’ shell compartment, can therefore serve as a good measure for the emulsions’ stability. Separating a two-phase system generates a large surface area per drop, leading to a high excess Gibbs energy per drop and thus to a tendency in the direction of decreasing the Gibbs energy through several types of interaction patterns such as flocculation, coalescence, Ostwald ripening and creaming [23–25]. Flocculation, for example, would be manifest in the shells’ microstructure as clustered pores separated by thin polymer walls, whereas coalescence would be apparent from the larger average pore size and a greater deviation in pore sizes (higher standard deviation). The process-structure release profile effects of our system are discussed below.

4.1. Effect of drug type on shell microstructure and drug-release profile

The presence of a hydrophilic drug in the emulsion’s aqueous phase modifies the emulsion’s hydrophobic-hydrophilic balance. An increase in the drugs’ charge or concentration corresponds to an increase in the interfacial tension between the aqueous and organic phases and is therefore expected to result in lower emulsion stability. This phenomenon was confirmed even when a moderate drug loading of 5% w/w was used. The pore shape ranged from completely round pores separated by clear polymer boundaries for ceftazidime-loaded samples (Fig. 2 c) to larger, somewhat irregular pores for gentamicin-loaded samples (Fig. 2 e). It should be noted that gentamicin, a highly charged polycation, displays the largest charge (+3.5, pH 7.4 [26]). Differences in shell morphology were thus obtained due to differences in the drugs’ hydrophilicity and charge.

![Fig. 7](image)

Fig. 7. Demonstration of bioactivity of the drug released from a composite fiber-based dressing against relevant bacterial strains: (a) *Pseudomonas aeruginosa*, (b) *Staphylococcus aureus* and (c) *Staphylococcus epidermidis*. Ceftazidime-loaded dressings demonstrate clear inhibition zones around them (left), whereas unloaded controls do not (right).

![Fig. 8](image)

Fig. 8. Tensile stress-strain curves of polyglyconate fibers. Fiber 1, original Maxon™ suture; Fiber 2, surface-treated core fiber; Fiber 3, reference formulation core-shell fiber structure containing mafenide (total diameter is considered); Fiber 4, reference formulation core-shell fiber structure containing mafenide (effective diameter is considered).

<table>
<thead>
<tr>
<th>Fiber type</th>
<th>Strength (MPa)</th>
<th>Modulus (MPa)</th>
<th>Ultimate strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Maxon™ suture</td>
<td>771 ± 63</td>
<td>478 ± 54</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>2 Treated Maxon™</td>
<td>503 ± 44</td>
<td>476 ± 39</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>3 Composite fiber A*</td>
<td>294 ± 18</td>
<td>233 ± 18</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>4 Composite fiber B**</td>
<td>487 ± 32</td>
<td>371 ± 14</td>
<td>42 ± 1</td>
</tr>
</tbody>
</table>

* Composite fiber A: evaluation based on the total fiber cross-sectional area.
** Composite fiber B: evaluation based on the effective fiber cross-sectional area.

a Statistically different (P < 0.05) from the Maxon™ suture.
b Statistically different (P < 0.05) from the surface-treated Maxon™ suture.
The adverse effect attributed to the drugs’ physiochemical properties was investigated by the introduction of various surfactants. Surfactants located at the interface between the aqueous phase and the organic phase reduce the interfacial tension between the two phases and act as emulsion stabilizers. Greater uniformity and a reduced pore size ($P < 0.05$) were observed when albumin was introduced into the formulation (Fig. 2). All three drugs were very similar in form (Fig. 2b, d and f), with pore sizes converging to 1–1.3 μm, thus suggesting that albumin is an effective surfactant for stabilizing our system. Albumin may contribute to the achievement of more than merely a stabilizing effect, since its ability to bind various drugs through specific interactions is well-known. Albumin, which contains approximately 100 acidic groups, 86 basic groups and 56% hydrophobic residues, is considered the predominant drug-binding protein in the body [27]. Its interaction with acidic or basic drugs by Van der Waals dispersion forces, hydrogen bonds and ionic interactions [28] may be utilized to counteract drug depletion. Attempts to employ this principle in albumin-sealed vascular grafts may be utilized to counteract drug depletion. Attempts to improve the release kinetics were based on modifications expected to increase restriction of the drug’s diffusion out of the shell. An increase in the O:A phase ratio did not significantly affect pore size, although it reduced the shells’ porosity. Such decrease in porosity was expected, as higher O:A ratios were attained by reducing the dispersed aqueous fraction of the emulsion (and subsequent pore fraction of the freeze-dried shell) whilst maintaining the same oil fraction. Samples with a high O:A phase ratio, e.g. 12:1, demonstrated relatively large polymeric domains between pores and less pore connectivity (Fig. 3b) compared to the reference sample with an O:A phase ratio of 6:1 (Fig. 3a), which creates a barrier for the diffusion of drug molecules. Pore connectivity in particular has a prominent effect on the burst release of the antibiotics. In view of the fact that the hydrophilic antibiotic is located within the shell’s pores, a highly interconnected structure poses almost no restriction to outward drug diffusion once water penetrates the shell and the drug’s release is therefore most probably governed by the rate of water penetration into the shell. Samples with O:A phase ratios up to 8:1 typically demonstrate great pore connectivity and their in vitro release patterns are almost identical (Fig. 6a), displaying a burst release of approximately 95%. Conversely, the porous shell structures attained for higher O:A phase ratios, e.g. 10:1 and 12:1, display reduced pore connectivity and a lower pore fraction (Table 2), resulting in a significant ($P < 0.05$) half-fold decrease in the burst release of antibiotics to approximately 45% (Fig. 6a). It can therefore be concluded that an increase in the O:A phase ratio to a value of 10:1 or higher results in a significant decrease in the burst release and a more moderate release profile can therefore be attained during the first 3 days.

4.3. Effect of polymer content on shell microstructure and drug-release profile

An increase in the emulsion’s polymer content resulted in a dramatic decrease in the burst release and a more moderate release profile. Burst release values of 95%, 81% and 29% were obtained for shell structures with polymer contents of 15%, 17.5% and 20%, respectively. A higher polymer content in the organic phase results in denser polymer walls between pores after freeze-drying (Fig. 3c) and therefore results in better constraint on the release of drugs out of pores. It should be noted that samples containing a 20% polymer content exhibited a three-phase release pattern: an initial burst release, a continuous release at a declining rate during the first 2 weeks until release of 50% of the encapsulated drug, followed by a third phase of release of a similar nature reaching 99% release after 50 days. The second phase of release is governed by diffusion, whereas the third phase is probably governed by degradation of the host polymer which enables trapped drug molecules to diffuse out through newly formed elution paths. In most of the cases described thus far, drug release was governed primarily by diffusion, since almost the entire amount of drug was released before polymer degradation would have in fact been able to contribute to its release. Thus, when drug diffusion out of the shell is restricted, as in the case of a high polymer content, and a considerable amount of drug still remains within the shell, polymer degradation will contribute to further release the antibiotics, which leads to an additional release phase.

4.4. Effect of polymer MW on shell microstructure and drug-release profile

An increase in the polymer’s MW from 100 to 240 kDa resulted in the most prominent effect on the shell microstructure (Fig. 3d). The porosity of the shell in this case was reduced to only 16% (Table 2) and pore size decreased to approximately 0.5 μm. Since high viscosity increases the shear forces during the process of emulsification and also
4.5. Tensile mechanical properties

Maxon™ sutures were chosen as carrying fibers as they combine relatively high tensile strength with good flexibility. Furthermore, their degradation rate is lower than that of the porous PDLGA coating. The mechanical properties we report for the Maxon™ sutures are in good agreement with previous studies [32].

The stress–strain curve of the surface-treated sutures closely followed that of the original fiber (Fig. 8), whereas the treated fibers failed at a lower stress (503 MPa) compared to the original fibers (771 MPa), probably due to cracks and imperfections caused at the fibers’ surface as a result of the treatment. This is still acceptable. The deterioration in mechanical properties can, however, be avoided in the future if a carrying fiber is extruded specifically for this application instead of using a commercial suture material which requires surface treatment. The results of the mechanical testing of the coated fibers demonstrate that the process of fiber coating, which includes exposure to the emulsion, quenching in liquid nitrogen and freeze-drying, results in an actual decrease in tensile strength and modulus (Table 3). Nevertheless, our composite fibers loaded with 5% drug exhibited a tensile strength of 487 MPa. The insignificant decrease in tensile strength, compared to the treated fiber (Table 3), shows that the fiber remains strong and still possesses superior mechanical properties compared to monolithic or reservoir fibers. For example, the recently reported monolithic polycaprolactone fibers containing 5-18% gentamicin exhibited tensile strengths of 4.2–7.4 MPa [7], a decrease of 4–45% compared to the unloaded fiber. Our antibiotic-eluting core–shell fiber structures can therefore in addition to wound dressings also be used for other biomedical applications which require good initial mechanical properties, e.g. as meshes for hernia repair.

5. Conclusions

New antibiotic-eluting bioresorbable core–shell fiber structures were developed and studied. These structures were composed of a polyglyconate core and a porous PDLGA shell loaded with the antibiotics mafenide acetate, ceftazidime and gentamicin. These structures are designed for use as basic elements of drug-eluting wound dressings. The shell structures were prepared using the technique of freeze-drying an inverted emulsion. This technique is advantageous because it results in a combination of good mechanical properties with the desired drug-release profile and preservation of the drug’s activity. The fiber investigation focused on the effects of the emulsion’s composition (formulation) on the shell microstructure and on the resulting drug-release profile from the fibers. The mechanical properties were also studied. In general, porous “shell” structures (mean porosities of 16–82% and mean pore sizes of 0.5–5 μm) were obtained with good adhesion to the core fiber. Albumin was found to be an effective surfactant for stabilizing the inverted emulsions and to have a beneficial holdup effect on the release of hydrophilic antibiotic drugs, especially mafenide, probably through specific interactions.

The release profiles commonly exhibited an initial burst effect accompanied by a decrease in release rates with time over periods ranging from several days to 50 days, depending on the formulation. Higher O:A ratios, polymer contents and MWs reduced the burst release of antibiotics from the fibers and prolonged their release due to changes in the shell structure. A higher MW and polymer content demonstrated a larger effect on the microstructure and release profile than the O:A phase ratio. In practice, a wound dressing can be woven from a combination of several types of fibers to create a resultant release profile which is the product of several release profiles or drug types. The diverse profiles achieved in this study with higher and lower burst release rates and with varying elution spans may serve as a good basis for further in vivo examination of the fibers in order to create the ideal profile for a particular wound healing application.

Acknowledgments

We would like to thank Ms. Moran Shohat, Tel-Aviv University, for her assistance with the drug-release studies. The authors are grateful to the Israel Science Foundation (ISF, Grant No. 1312/07), the Israel Ministry of Health (Grant number 3-3943) and to the Ela Kodesz Foundation, Tel-Aviv University, for supporting this research.
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