Hybrid wound dressings with controlled release of antibiotics: Structure-release profile effects and in vivo study in a guinea pig burn model

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A B S T R A C T

Over the last decades, wound dressings have evolved from a crude traditional gauze dressing to tissue-engineered scaffolds. Many types of wound dressing formats are commercially available or have been investigated. We developed and studied hybrid bilayer wound dressings which combine a drug-loaded porous poly(o–lactic-co-glycolic acid) top layer with a spongy collagen sublayer. Such a structure is very promising because it combines the advantageous properties of both layers. The antibiotic drug gentamicin was incorporated into the top layer for preventing and/or defeating infections. In this study, we examined the effect of the top layer’s structure on the gentamicin release profile and on the resulting in vivo wound healing. The latter was tested on a guinea pig burn model, compared to the neutral non-adherent dressing material Melolin® (Smith & Nephew) and Aquacel® Ag (ConvaTec). The release kinetics of gentamicin from the various studied formulations exhibited burst release values between 8% and 38%, followed by a drug elution rate that decreased with time and lasted for at least 7 weeks. The hybrid dressing, with relatively slow gentamicin release, enabled the highest degree of wound healing (28%), which is at least double that obtained by the other dressing formats (8–12%). It resulted in the lowest degree of wound contraction and a relatively low amount of inflammatory cells compared to the controls. This dressing was found to be superior to hybrid wound dressings with fast gentamicin release and to the neat hybrid dressing without drug release. Since this dressing exhibited promising results and does not require frequent bandage changes, it offers a potentially valuable concept for treating large infected burns.

1. Introduction

1.1. Wound dressings

Over the last decades, wound dressings have evolved from the crude traditional gauze dressing to tissue-engineered scaffolds. Many types of wound dressing formats designed for specific wound-healing functions are commercially available or have been investigated [1]. A wound dressing should ideally provide an optimal healing environment which enables rapid healing. It should maintain a moist environment at the wound surface, allow gas exchange, act as a barrier to microorganisms and remove excess exudates [2]. Some modern dressings are therefore designed according to the well-accepted bilayer structure in order to provide a better healing environment compared to homogeneous films. The upper dense layer is designed to control moisture transmission, prevent bacterial penetration and afford mechanical protection to the wound. The lower spongy layer is designed to absorb wound exudates, smoothly adhere to the wet wound bed and accommodate newly formed tissue [3,4].

Based on these principles, various bilayer structures in which both layers are based on natural [5–8] or synthetic polymers [4] have been designed over the years. Although natural polymers have the advantage of being similar or identical to macromolecules in our body, they suffer from the disadvantage of undergoing rapid
in vivo degradation by proteases [9]. The incorporated drug is rapidly diffused out by a combination of diffusion and natural enzymatic breakdown of the protein [5,6]. However, dressings based on synthetic polymers do not fully promote cell adhesion and proliferation due to their inherently inert surface chemistry [10], and do not allow smooth adherence to the wound bed, which may lead to bacterial infection.

Hybrid bilayer wound dressings are very promising, since they enable combining the advantageous properties of a natural sublayer and a synthetic top layer. Such a design is very challenging, due to the different nature of synthetic and natural polymers, which leads to difficulties in binding between them.

Controlled release of bioactive agents from wound dressings has also been studied. Much attention has focused on wound dressings that provide an inherent antimicrobial effect by eluting germicidal components in order to prevent bacterial infection [5,11,12]. To date, not enough research has focused on local release of antibiotics.

We have recently reported the development of hybrid bilayer wound dressings which combine a drug-loaded porous poly(α,ω-lactic-co-glycolic acid) (PDLGA) top layer with a spongy collagen sublayer [13]. Ibuprofen and bupivacaine were incorporated into the top layer for pain management. The top layer can be tailored to produce the desired drug-release kinetics as well as to control moisture evaporation from the dressing. The spongy collagen layer is designed to maintain high absorption of wound exudates and to accommodate newly formed tissue. In that paper [13] we also reported a simple methodology for integrating the collagen sponge layer with a synthetic PDLGA layer into a unique hybrid structure, and the characteristic features of the newly designed dressings in terms of mechanical and physical properties as well as release profiles of analgesic drugs.

1.2. Burn infections and guinea pig model

Burn wound infections are among the most important and potentially serious complications that occur during the acute period following burn injury. Burn wound surfaces are sterile immediately after the thermal injury. However, colonization with autogenous microorganisms or through contact with the contaminated environment generally occurs within 48 h [14,15]. Inadequate wound perfusion restricts migration of the host’s immune cells and delivery of antimicrobial agents to the wound, thus limiting the effectiveness of systemic treatments. The local antibiotic concentration may be insufficient and may lead to bacterial resistance. Application of a topical antimicrobial agent on the open burn wound surface can substantially reduce the microbial load and risk of infection [16]. However, such treatment requires frequent changes of the dressing material, causes inconvenience to the patient and places a financial burden on the healthcare system. Uncomplicated skin infections account for almost 200 million annual physician-office visits in the US, and treatment of these infections is estimated to cost over $350 million annually [17].

The advent of new generations of drugs, topical agents and synthetic dressings necessitates the use of a proper experimental model for evaluating their potential beneficial effects on the healing of burn wounds, and in particular the three main components of the burn wound healing process: epithelialization, contraction and scar formation [18]. If the agent or device is capable of reversing the microcirculatory stasis in the stasis zone via pharmacological, biochemical or physical mechanisms, deepening of the burn wound is prevented and spontaneous healing can be expected. A deep partial skin thickness burn may thus be prevented from converting into a full thickness injury which requires skin grafting. The guinea pig is often used as a dermatological and infection model [18–21]. Research on guinea pigs has included topical antibiotic treatment [22], delivery of delayed-release antibiotics [23], and investigation of wound dressing materials [24,25]. A deep partial skin thickness burn is an excellent wound model for the evaluation of wound healing, not only for contraction and epithelialization of the peripheral area such as in third-degree burns, but also for evaluation of the recovery of skin appendages which serve as the main source for the re-epithelialization which completes the healing process. The metabolic response to severe burn injury in guinea pigs is very similar to that of humans [26]. Furthermore, bacterial colonization and changes within the complement component of the immune system in human burn victims is analogous to guinea pigs affected by severe burns [20].

In the current study, we report the microstructure of our new hybrid bilayer wound dressings, the controlled release profiles of the antibiotic drug gentamicin from these wound dressings and their effect on the healing of burn wounds. The guinea pig burn model described above was used to evaluate the effectiveness of our novel hybrid antibiotic-eluting wound dressing, compared to the neutral non-adherent dressing material Melolin® (Smith & Nephew) and Aquacel® Ag (Convatec). Our new antibiotic-eluting wound dressing platform is advantageous over current popular dressing materials that provide controlled release of silver ions as the antibacterial agent, since these may have toxic effects on cells and may delay wound healing. It is important to note that biodegradable drug-eluting wound dressings which present an alternative to silver ion-eluting dressings are currently not available on the market.

2. Materials and methods

2.1. Materials

Synthetic polymer: Poly(α,ω-lactic-co-glycolic acid) with a copolymeric ratio of 50% lactic acid and 50% glycolic acid (50/50 PDLGA), inherent viscosity (i.v.) = 0.65 dL/g (in CHCl₃ at 30 °C) (molecular weight approximately 50 kDa), Absorbable Polymer Technologies, Inc., USA.

Natural polymer: Collagen-klee® 10 × 10 × 0.5 cm (a natural resorbable spongy membrane from porcine dermis consisting of a minimum of 96.75% native collagen type 1), Medical Biomaterials Products GmbH, Germany (10105).

Drug: Gentamicin sulfate (Sigma, G-1264).

Reagents: Reagent kit (8-1P31-25) and calibration kit (8-1P31-01) for the analysis of gentamicin concentrations were purchased from Sigma–Aldrich, Rehovot, Israel.

2.2. Hybrid wound dressing preparation

The preparation of the hybrid wound dressing is based on two stages. First, an inverted emulsion loaded with the drug molecules is prepared. The bilayer hybrid wound dressing is then prepared, based on the freeze-drying of an inverted emulsion, as follows:

(a) Preparation of the inverted emulsion

The aqueous phase of the inverted emulsion was based on double-distilled water and the drug gentamicin was included in it. The organic phase of the inverted emulsion contained 15% (w/v) of 50/ 50 PDLGA dissolved in chloroform. Homogenization of the two phases was performed for 90 s at 16,000 RPM using a Kinematica PT-2500 E Polytron homogenizer. Four formulations were used in the current study to produce the top layer of the wound dressing materials:
I. 6:1 organic:aqueous (O:A) phase ratio. This emulsion served as a reference and therefore did not contain gentamicin. It is termed “Ref”.

II. 6:1 organic:aqueous (O:A) phase ratio, and an aqueous phase which contained 10% (w/w) gentamicin and 1% (w/v) BSA as surfactant. This formulation is termed BSA.

III. 12:1 organic:aqueous (O:A) phase ratio, and an aqueous phase which contained 10% (w/w) gentamicin and 1% (w/v) BSA as surfactant. This formulation is termed BSA2.

IV. 12:1 organic:aqueous (O:A) phase ratio, and 1% (w/v) sorbitan monooleate (Span 80) was added to the organic solution as surfactant. The aqueous phase contained 10% (w/w) gentamicin. This formulation is termed SPAN.

The parameters of all four formulations are summarized in Table 1. Based on the formulation parameters given above, the calculated drug load of each wound dressing is 3.6 mg/cm².

(b) Preparation of the bilayer structures

An aluminum tube with rounded and homogeneously dispersed holes on its lower surface (D = 5 cm), used as a dip-coating instrument, was connected to a vacuum source to hold the collagen sponge. The sponge was then dip-coated in a fresh inverted emulsion for a few seconds and then immediately frozen in a liquid nitrogen bath. The samples were then placed in a pre-cooled (−105 °C) freeze-dryer (Virtis 101 equipped with a nitrogen trap) and freeze-dried to preserve the microstructure of the emulsion-based structures. Drying was performed in two stages: The freeze-dryer chamber pressure was reduced to 100 mTorr while the temperature remained at −105 °C. After 5 h, a hot plate was turned on to −30 °C overnight. The condenser was then turned off 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 dried samples were stored in desiccators until use. Our previous study shows that the freeze drying process does not affect antibiotics activity [12].

2.3. Morphological characterization

The morphology of the wound dressing structures was observed using an environmental scanning electron microscope (ESEM) with a Quanta 200 FEG at a high vacuum mode and accelerating voltage of 10 kV. The cryogenically fractured surfaces were Au-sputtered prior to observation. The mean pore diameter (n = 150 pores from 10 different ESEM fractographs) and porosity of the observed morphologies were analyzed using the Sigma Scan Pro software and statistics were calculated using the SPSS 18 software. The area occupied by the pores was calculated for each fractograph in order to evaluate the porosity of the samples. Porosity was determined as the area occupied by the pores divided by the total area.

Table 1

<table>
<thead>
<tr>
<th>The formulation</th>
<th>O:A phase ratio</th>
<th>Surfactant</th>
<th>Gentamicin content</th>
<th>Burst release (%)</th>
<th>Porosity (%)</th>
<th>Pore diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref (reference)</td>
<td>6:1</td>
<td>BSA</td>
<td>0</td>
<td>None</td>
<td>68 ± 4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>BSA and also “fast gentamicin release”</td>
<td>6:1</td>
<td>BSA</td>
<td>10% w/w</td>
<td>38 ± 4</td>
<td>63 ± 4</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>BSA2</td>
<td>12:1</td>
<td>BSA</td>
<td>10% w/w</td>
<td>18 ± 2</td>
<td>45 ± 2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>SPAN and also “slow gentamicin release”</td>
<td>12:1</td>
<td>BSA 80</td>
<td>10% w/w</td>
<td>8 ± 2</td>
<td>35 ± 5</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

* All formulations are based on 50/50 PDLGA with polymer contents of 15% w/v in the organic phase.
** Burst release was measured during the first 6 h of release.

2.4. In vitro drug-release studies

Small disk-shaped (D = 1.5 cm) pieces (triplicates) were immersed in phosphate-buffered saline (PBS, pH 7.0) and kept at 37 °C for 84 days in order to determine the various drug release kinetics from these structures. The release studies were conducted in closed glass vessels containing 2.5 ml PBS. Sodium azide (0.02 %) was added in order to prevent microbial contamination. The medium was removed (completely) periodically at each sampling point (6 h, 1, 2, 3, 7, 14, 21, 28, 35, 42, 56, 70, 84 days), and fresh medium was introduced. Residual drug recovery from wound dressings was carried out using a Trypsin A solution to dissolve the remaining collagen sponge. Next, 1 ml of methylene chloride and 2 ml of DDW were used in order to dissolve the hydrophilic drug residues.

Determination of the medium’s gentamicin content was carried out using the Architect i2000SR (Abbot Laboratories) according to the manufacturer's instructions. This machine enables determination of the gentamicin concentration based on a chemiluminescent microparticle immunoassay (CMIA). An Architect iGentamicin Reagent kit (IP31) was purchased for the analysis. The measurable concentration range without dilution is 0.0–10.0 mg/mL. Higher drug concentrations were measured after carrying out manual dilutions.

2.5. In vivo study

(a) Procedure and treatment groups

20 female guinea pigs (Arlan, NL) were allocated for the study after authorization by the Animal Care and Use Committee (authorization IL-092-09-2012). The animals (average weight 300 ± 30 g) were housed in separate cages, and after 5 acclimatization days were randomly divided into 5 groups. All animals were anesthetized by intramuscular injection of ketamin (40–50 mg/kg) and xylazine (4–5 mg/kg). The animal’s skin was shaved and a depilatory cream (Orna 19, Alpha Cosmetica, Israel) was applied to complete hair removal. Two standardized deep-second-degree burns were inflicted on the back of each animal on both sides of the spine according to a validated method described by Kaufman et al. [18]. Iron templates (circle, D = 40 mm) were immersed in water preheated to 75 °C and then placed in a perfect contact with the animal’s skin for exactly 5 s by applying light pressure. The extent of the burn was traced onto a transparent paper as a reference for later follow-up.

Ten minutes after the infliction of the burns, each animal was seeded with 0.5 mL broth containing 5 × 10^6 CFU/mL *Pseudomonas aeruginosa* using a micropipette. Each group was then treated with the relevant treatment option, as follows.

Group 1 (Melolin) was treated with a neutral non-adherent dressing material (Melolin®, Smith & Nephew). Melolin® is composed of three layers: a low adherent perforated film, a highly absorbent cotton/acrylic pad and a hydrophobic backing layer. According to the manufacturer, it allows for rapid drainage of wound exudate, thus reducing trauma to the healing tissue. The

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dressing was placed directly on the burn, and was secured by an elastic adhesive bandage (Tensoplast™, Smith & Nephew).

Group 2 (Aquadac Ag) was treated with Aquadac Ag (ConvaTec). Aquadac Ag consists of hydrofiber and ionic silver which has antimicrobial activity. Ionic silver is released in a controlled manner. This dressing is commonly used for burns and chronic wounds, and in this study was used as a control.

Group 3 (REF) was treated with our hybrid dressing, derived from the reference (formulation I, see also Table 1), which did not contain antibiotics. A round disk slightly larger than the burn area (D = 45 mm) was placed directly on the burn, covered with Melolin™, and secured as described above. This dressing material was tested in order to evaluate the effect of the dressing's texture, materials, and degradation on the wound healing process.

Groups 4 and 5 (BSA and SPAN, respectively) were treated with our hybrid dressings derived from formulations II (BSA) and IV (SPAN), respectively (see Table 1), which enabled fast and slow gentamicin release (see the gentamicin release profile results below). The dressing materials were placed and secured as described in group 3. These dressing materials were tested in order to evaluate the effect of antibiotic release kinetics on the wound healing process.

Each animal was placed in an individual cage with food ad libitum and allowed to recover. The animals received analgesic treatment prior to the procedure and during 5 consecutive days (ketoprofen 5 mg/kg, subcutaneous).

(b) Post mortem examination

Animals were anesthetized after 12 days, and the dressing materials were removed. We chose 12 days, which based on our experience is not long enough for complete healing and thus enables observation of differences in the wound healing rate between the studied groups. The closed (epithelialized) wound area and open (non-epithelialized, bleeding) wound area were traced on a transparent paper, 1 cm² biopsies were taken from the center of each burn wound and immediately fixed in phosphate-buffered formalin. Sections were stained with hematoxylin and eosin (H&E), observed and photographed under 20× and 100× power, using an Olympus BH2 microscope. Wound healing analysis was conducted in a blinded manner by two separate evaluators using a semi-quantitative grading system. Sections were evaluated based on structure and content. The wound-healing criteria included epithelialization, epidermis–dermis attachment, angiogenesis, adnexa (hair follicles, sweat glands and sebaceous glands), dermis thickness, mononuclear cells infiltrate and collagen. Grading was between 0 and 5: 0 – absence, 1 – minimal presence, 2 – minimal to moderate presence, 3 – moderate presence, 4 – moderate to extensive presence, and 5 – extensive presence.

2.6. Statistics

In vitro drug release study: statistics were calculated using the SPSS 15 software. All data are expressed as mean ± standard deviation (SD).

Animal studies: means and standard error of mean (SEM) were calculated. Differences between means were analyzed for statistical significance using a one-way ANOVA with the Tukey–Kramer multiple comparisons posttest (SPSS version 17.0). For the scoring evaluation, statistical significance was determined using Mann–Whitney U test. p values ≤0.05 were considered significant.

3. Results

In the current study, the microstructure of the upper PDLGA layer was observed and its effect on the release profiles of gentamicin was elucidated. The effects of the gentamicin release profile on the healing process of burn wounds were also studied.

3.1. The hybrid wound dressing structure and in vitro gentamicin release profile

As mentioned, our new hybrid wound dressings combine a porous drug-eluting PDLGA top layer with a sponge collagen sublayer which is in contact with the damaged skin. A photograph of this hybrid wound dressing is presented in Fig. 1a and an ESEM micrograph showing the layers in detail is presented in Fig. 1b. In this unique structure, both layers are bonded by a third interfacial layer (Fig. 1b). Collagen degradation results (not presented here) show that samples lost about 40% of their initial weight after the first 24 h in an aqueous medium. Minor changes in the weight loss of the collagen occurred during the following 2 months.

The main challenge in designing a device for the release of low molecular weight hydrophilic bioactive agents (such as the antibiotics used in the current study) is to overcome their rapid discharge from the device. A drug-eluting bilayer structure is even more challenging, especially when the drug is incorporated within the top layer and its discharge from the device also depends on the swelling rate of the lower layer. We used a non cross-linked collagen sponge with high porosity and swelling rate in both of our systems so that it would not decrease the drug release rate from the upper layer to the wound bed. When we compared the drug release profile from our hybrid systems to that obtained from the upper layer only, we did not observe any significant changes.

The freeze-drying of inverted emulsions technique was used to produce the drug-eluting top layer. This technique was proven to be efficient for producing a system which enables prolonged release of active agents in a controlled manner, which also enabled inhibition of bacterial strains for at least 2 weeks, when antibiotic drugs are released. It is unique in its ability to preserve a liquid structure in the solid state. Thus, the microstructure of the porous matrix can be customized through modifications of the emulsion’s formulation parameters, which affect drug release kinetics.

Three different emulsion formulations loaded with gentamicin were used in the current study, as listed in Table 1. These three formulations yielded different resultant upper layer microstructures. The microstructure of the BSA sample is highly porous (Fig. 1c), with an average porosity of 63 ± 4% and a pore diameter of 1.4 ± 0.3 μm (Table 1). An increase in the emulsions’ O:A phase ratio from 6:1 to 12:1 (formulation BSA2) resulted in larger polymer domains between pores, less pore connectivity, and a lower porosity of 45 ± 2% (Fig. 1d and Table 1). However, it did not affect the pore size, which remained 1.4 ± 0.3 μm. When the surfactant BSA which was used to stabilize the emulsion was replaced with Span 80 (formulation SPAN) for the same O:A phase ratio (12:1), the mean pore size decreased to 1.1 ± 0.3 μm and the porosity decreased to 35 ± 5% (Fig. 1e and Table 1).

The cumulative release of antibiotics from dressings based on emulsion formulations containing 10% (w/w) gentamicin stabilized with BSA or Span is presented in Fig. 2. It should be noted that the various emulsion formulations used in the current study enabled the achievement of a broad range of drug release profiles from the hybrid dressings. The BSA sample typically demonstrated a relatively high burst release of gentamicin (38 ± 4%), followed by a gradual release in a decreasing rate over time with 80% release of the encapsulated drug within 4 days. This was called the fast release sample. In contradistinction, the SPAN formulation demonstrated better restraint over gentamicin release, with a much lower burst release (8 ± 2%) and a longer overall release of gentamicin (84 days). This was called the slow release sample. The BSA2 formulation demonstrated an intermediate drug release behavior, with a medium burst release followed by a medium release rate.
3.2. In vivo study

We used three dressing materials: one as the reference without gentamicin (Ref) and the other two with fast release (BSA) and slow release (SPAN) of gentamicin (Fig. 2). Melolin\(^\text{\textregistered}\) and Aquacel\(^\text{\textregistered}\) Ag groups served as controls.

3.2.1. Wound closure

Second-degree burns in guinea pigs were used as a wound-healing model for testing our novel hybrid wound dressing. Pseudomonas was applied topically immediately after the infliction of the burns in order to mimic burn contamination that typically occurs in patients with burns.

Twenty guinea pigs were included in the study and were divided into five groups: Melolin\(^\text{\textregistered}\) control group, Aquacel\(^\text{\textregistered}\) Ag control group, hybrid dressing without antibiotic (Ref), hybrid dressing with slow release of gentamicin (SPAN) and hybrid dressing with fast release of gentamicin (BSA) (Fig. 2). Representative photographs were taken at the study endpoint (12 days) (Fig. 3). The closed (epithelialized) wound area and the open (non-epithelialized) wound area were traced on a transparent paper 12 days following burn creation. The degree of healing was calculated by the percentage of the epithelialized (closed) area on the 12th day compared to the total burn area on the first day (Fig. 4A). On the 12th post-burn day, 8% ± 3.9 of the wounds that were dressed with Melolin\(^\text{\textregistered}\), 12% ± 4.3 of the wounds in the Aquacel\(^\text{\textregistered}\) Ag group, 11% ± 5.1 of the wounds in the hybrid reference group, and 8% ± 3.1 of the wounds in the BSA group were epithelialized, whereas the closed area of the hybrid group with slow gentamicin release (SPAN) reached 28% ± 8.5 (Fig. 4A). Significantly improved
wound closure was obtained only in the hybrid slow-release dressing (SPAN) \( (p < 0.05) \). The differences in wound closure in the other studied groups were not significant.

### 3.2.2. Wound contraction (1-area 12 days/area 1 day)

Wound contraction was calculated as 1 minus the total wound area (epithelialized and non-epithelialized) divided by the original area subjected to burn injury. A significant reduction in the contraction rate was observed in the group treated with the slow gentamicin release dressing (SPAN) compared to the group treated with Melolin\(^8\) \( (31\% \pm 3.2, 53 \pm 2.5, \text{respectively, } p < 0.001) \). In contraposition, Aquacel\(^8\) Ag resulted in the highest contraction rate \( (66\% \pm 3.3) \), even compared to the group treated with Melolin\(^8\) \( (p < 0.05) \). The other two hybrid groups (BSA and Ref) demonstrated a slight non-significant reduction in the contraction rate compared to the Melolin\(^8\) control group (Fig. 4B).

### 3.2.3. Histological evaluation

Animals were sacrificed 12 days following burn creation, and 1 cm\(^2\) biopsies were taken from the center of the wound and immediately fixed in phosphate buffered formalin. Two different observers examined the biopsies that were stained with H&E (Fig. 3), according to 7 criteria. The results (total scores) of the desired characteristics (angiogenesis, epidermis/dermis, epithelialization, dermis thickness, adnexa and collagen formation) are presented in Fig. 5. Fig. 6 shows each criterion separately. Fig. 7 presents the (undesired) mononuclear infiltration score which indicates inflammatory cells.

The slow gentamicin release group (SPAN) and the reference group without antibiotics (Ref) demonstrated slightly superior total scores compared to the other groups (Fig. 5). More particularly, they demonstrated improvement in epidermis–dermis attachment and a significant improvement in the epithelialization parameters compared to the group treated with Melolin\(^8\) \( (p < 0.05, \text{respectively}) \).
Fig. 6). All our hybrid wound dressings (SPAN, BSA and Ref) demonstrated a significant decrease in mononuclear infiltration compared to the control groups (Melolin® and Aquacel® Ag (p < 0.05) (Fig. 7).

4. Discussion

In our hybrid wound dressings, the spongy collagen layer is designed to absorb wound exudates, smoothly adhere to the wet...
wound bed as well as to accommodate newly formed tissue. The advantages of collagen-based dressings over other systems are due to their unique features such as weak antigenicity, biodegradability and superior biocompatibility [6,27]. Such systems have been reported to perform better than conventional and synthetic dressings in accelerating granulation tissue formation and epithelialization [6,28]. The porous synthetic PDLGA top layer is designed to control moisture transmission, prevent bacterial penetration as well as to act as a drug reservoir. PDLGA is a mechanically reliable polymer that has been proven to perform well in various implants and long-term drug delivery systems [29,30]. Taken together, both materials synergistically produce properties which are not available in the individual constituent materials. A similar concept was used in several commercially available dressings, such as Integra® which uses a silicone upper layer and a collagen-glycosaminoglycan sublayer. It is important to note that contrary to other systems, all of the structural constituents in our new systems are biodegradable. Dressings made from these constituents therefore do not need to be removed from the wound surface once they have fulfilled their role. Furthermore, none of the currently available bilayer wound dressings release drugs to the wound site in a controlled manner.

One of the challenges in fabricating a bilayered structure so that it can fulfill its function is to ensure adhesion between the two distinct layers. Integration between a synthetic and a natural polymer is challenging due to their different structural and chemical properties. Contrary to previously described methodologies for chemically combining natural and synthetic polymers [5,31], we report a simple dip-coating technique for physically binding between the natural polymer collagen and the synthetic polymer PDLGA, which enables the penetration of the inverted emulsion into the collagen pores when vacuum is used. This results in an interface between the collagen and PDLGA porous layer in the solid state, which actually behaves like an interphase in which both materials are mechanically mixed and therefore the two layers are well held together. Superior mechanical properties such as tension, as well as physical properties (water uptake, water vapor transmission rate, etc.), were obtained and are described elsewhere [13].

4.1. The microstructure of the upper layer and its effect on the gentamicin release profile

In the current study, we focused on three drug-eluting upper layer formulations which display distinctly different microstructural features (Fig. 1 and Table 1). The effect of the O:A phase ratio was examined on formulations containing BSA as surfactant. As expected, a higher O:A phase ratio, i.e., lower aqueous phase quantity of the inverted emulsion, resulted in a smaller porosity of the solid structure. However, both microstructures were homogenous and were characterized by a similar average pore size. The stabilization effect of Span 80 was even higher than that obtained using BSA, and therefore resulted in a smaller pore size (Table 1).

The advantage of the freeze-drying of inverted emulsions technique used in our study is that the drug is incorporated within a porous structure during the fabrication process, in order to obtain its release in a controlled desired manner. Our results show that the highly porous (63%) upper layer based on the BSA formulation (with an O:A phase ratio of 6:1) exhibited a relatively high burst release of antibiotics (38%) and 80% release of the encapsulated drug was released within 4 weeks. A finer microstructure with thicker polymer walls between pores thus enables slower diffusion of the hydrophilic antibiotic molecules to the surrounding.

4.2. In vivo study

The post-burn end-point was chosen to be twelve days. At this stage, the wounds in all groups demonstrated wound healing in the range of 8–28% of the original wound area. The slow gentamicin release hybrid treated group (SPAN) demonstrated a significant increase in the epithelialization rate (degree of healing) compared to the Melolin® control group, while the other groups demonstrated only a slight non-significant increase of the healing rate (Fig. 4A). This group (SPAN) also had the least wound contraction (31%, Fig. 4B). Wound contraction is an inherent part of the normal wound healing process, but may lead to disfigurement of the skin and poor esthetic results, as well as loss of the normal skin flexibility that may entail a functional disability.

A clear benefit for the slow release gentamicin dressing was also found in the histological parameters: epithelialization, dermal–epidermal junction (Figs. 5 and 6) and fewer inflammatory cells (Fig. 7). Nevertheless, the reference hybrid dressing without gentamicin (Ref) also had better epidermal–dermal junction, epithelialization and fewer inflammatory cells. This indicates that the collagen itself affords better conditions for wound healing than the other two control groups. The hybrid dressing with fast gentamicin release (BSA) did not show any advantage over the other groups in terms of angiogenesis, dermis thickness, adnexa and collagen formation (Fig. 6).

The commonly used local treatments contain antibacterial components. However, Siverol®, for example, requires daily or twice-daily changes of the dressing. Aquacel® Ag has antimicrobial activity, absorbs exudates from the wound, and does not need changing for up to 2 weeks. In this study, the relatively low degree of wound healing which was obtained for the Aquacel® Ag treatment is probably related to inhibition of epithelialization by the silver ion [32–34]. The slow gentamicin-release hybrid dressing is noticeably superior to the other investigated modalities. The next planned step in this study is to evaluate the effect of sterilization on the structure and properties and choosing the most appropriate conditions, so as to be able to keep desired properties. Then a broad animal study will be performed, before testing our new hybrid system on humans.

5. Summary and conclusions

Novel bioresorbable hybrid wound dressings which combine a synthetic (PDLGA) porous drug-loaded top layer with a spongy collagen sublayer were developed and studied. The top layer was prepared using the freeze-drying of inverted emulsions technique and contained the antibiotic drug gentamicin for controlled release to the wound site. Our investigation focused on the effects of the PDLGA's microstructure on the drug-release profile and on the resulting effect on wound healing, using a guinea pig burn model.

The top PDLGA layer exhibited a homogenous porous structure with mean pore sizes of 1–1.5 microns and porosity in a broad range of 35–68%, which enabled control over the antibiotic release profiles. Hybrid wound dressings with fast and slow gentamicin release rates, and the neat hybrid dressing (without drug), were evaluated in vivo in a contaminated burn wound model and compared to Melolin® and Aquacel® Ag controls. The hybrid wound dressing with slow gentamicin release significantly accelerated wound healing compared to all other tested wound dressings (28% vs. 8–12% after 12 days). Wound contraction was reduced significantly, and better quality scar tissue was formed. The biopsies demonstrated superior scores of the desired characteristics.
(angiogenesis, epidermis/dermis, epithelialization, dermis thickness, adnexa and collagen formation) in the slow gentamicin release group and the reference group without antibiotics compared to the other groups. All our hybrid wound dressings demonstrated a significant decrease in mononuclear infiltration compared to the control groups.

From a practical aspect, all of these lead to less pain to the patient, shorter hospitalization, and a better healing quality with less contraction. Thus, the current hybrid dressing material with slow gentamicin release shows promising results. It does not require bandage changes and offers a potentially valuable and economic approach for treating the life-threatening complication of burn-related infections.

Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figs. 1–7, are difficult to interpret in black and white. The full color images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2015.04.029.

References