Novel biodegradable composite wound dressings with controlled release of antibiotics: Results in a guinea pig burn model

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A B S T R A C T

Approximately 70% of all people with severe burns die from related infections despite advances in treatment regimens and the best efforts of nurses and doctors. Silver ion-eluting wound dressings are available for overcoming this problem. However, there are reports of deleterious effects of such dressings due to cellular toxicity that delays the healing process, and the dressing changes needed 1–2 times a day are uncomfortable for the patient and time consuming for the stuff. An alternative concept in wound dressing design that combines the advantages of occlusive dressings with biodegradability and intrinsic topical antibiotic treatment is described herewith. The new composite structure presented in this article is based on a polyglyconate mesh and a porous poly-(dl-lactic-co-glycolic acid) matrix loaded with gentamicin developed to provide controlled release of antibiotics for three weeks. In vivo evaluation of the dressing material in contaminated deep second degree burn wounds in guinea pigs (n = 20) demonstrated its ability to accelerate epithelialization by 40% compared to an unloaded format of the material and a conventional dressing material. Wound contraction was reduced significantly, and a better quality scar tissue was formed. The current dressing material exhibits promising results, does not require frequent bandage changes, and offers a potentially valuable and economic approach to treating the life-threatening complication of burn-related infections.

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1. Introduction

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 following the thermal injury. However, colonization with autogenous microorganisms or through contact with the contaminated environment, generally occurs within 48 h [1,2]. 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 [3]. However, such treatment requires frequent changes of the dressing material, causes inconvenience to the patient and comprises 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 [4].

Improved wound dressings that provide an inherent antimicrobial effect by eluting germicidal compounds have been developed to respond to the aforementioned problems associated with conventional topical treatments withointments and creams. Such dressings, e.g., Acticoat® (Smith & Nephew), Actisorb® (J&J) and Aquacel® (Convatec), usually incorporate silver ions as the active agent. They provide controlled release of silver ions through a slow but sustained release mechanism which helps avoid toxicity yet ensures delivery of a therapeutic dose to the wound. Despite frequent usage, it has been reported that silver ions are highly toxic to keratinocytes and fibroblasts and may delay burn wound healing if applied indiscriminately to healing tissue areas [5–8]. Furthermore, most of these dressing materials require frequent changes. Biodegradable dressings may successfully address this shortcoming, since they do not need to be removed from the wound surface.

The authors have recently developed and studied a new concept in wound dressing design which combines the advantages of occlusive dressings with biodegradability and intrinsic topical antibiotic treatment [9,10]. This new composite material is based on a polyglyconate mesh coated with a porous poly(α-lactic-co-glycolic acid) (PDLGA) matrix. It is designed to protect the wound until it is no longer needed, after which it dissolves into non-toxic end products by chemical (hydrolytic) degradation. The reinforcing mesh affords the necessary mechanical strength to the dressing, whereas the porous binding matrix is aimed to provide adequate moisture control and release of antibiotics, protect the wound bed from infection and promote healing. This material is porous, biodegradable and drug-eluting, thus avoiding the need for constant wound cleaning and redressing and should enable the body to better cope with healing and reduce patient pain and suffering. Biodegradable drug-eluting wound dressings which present an alternative to silver ion-eluting dressings are currently not available on the market.

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 main components of the burn wound healing process: epithelialization, contraction and scar formation [11]. 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 [11–14]. Research on guinea pigs has included topical antibiotic treatment [15], delivery of delayed-release antibiotics [16], and investigation of wound dressing materials [17,18]. 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, to serve as the main source for the re-epithelization, which completes the healing process. The metabolic response to severe burn injury in guinea pigs is very similar to that of the human post-burn metabolic response [19]. 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 [13]. Such a model was therefore used in the current study to evaluate the effectiveness of our novel composite antibiotic-eluting wound dressing.

2. Materials and methods

2.1. Wound dressing preparation

The antibiotic-eluting wound dressing preparation was based on the freeze-drying of inverted emulsion technique. The aqueous phase of the inverted emulsion was based on double distilled water, while the organic phase of the inverted emulsion contained 15% (w/v) of 50/50 poly(α-lactic-co-glycolic acid) (PDLGA), Absorbable Polymer Technologies, Inc., USA, dissolved in chloroform. Hand woven fibrous meshes (Monocryl TM 4-0, biodegradable polyglyconate monofilament sutures, Johnson & Johnson, Belgium) were dip-coated in freshly prepared inverted emulsions and then immediately frozen in a liquid nitrogen bath. The samples were then placed in a pre-cooled (−105 °C) freeze-dryer (Virtis 101) and freeze-dried in order to preserve the microstructure of the emulsion-based structures. Three emulsion formulations were used in the current study to create wound dressing materials:

I. Control wound dressing

6:1 organic: aqueous phase ratio was used and the aqueous phase also contained 1% (w/v) bovine serum albumin (BSA) as surfactant. This emulsion served as control and therefore did not contain gentamicin.

II. Wound dressing with fast gentamicin release

6:1 organic: aqueous phase ratio, and aqueous phase which contained 10% (w/w) gentamicin and 1% (w/v) BSA as surfactant.

III. Slow releasing emulsion

12:1 organic: aqueous 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.

2.2. Morphological characterization

The morphology of the wound dressing structures was observed using a Jeol JSM-6300 scanning electron microscope (SEM) at an accelerating voltage of 5 kV. Surfaces were Pd-sputtered prior to observation.

2.3. In vitro drug release studies

The composite wound dressings were immersed in phosphate buffered saline (PBS) at 37 °C for 28 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. The medium was removed (completely) periodically, at each sampling time (6, 12 h, 1, 2, 3, 5, 7, 14, 21 days), and fresh medium was introduced.

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 determination of the gentamicin concentration based on a polarization fluoroimmunoassay using fluorescein as a tracer. The measurable concentration range without dilution is 0.0–10.0 μg/mL. Higher drug concentrations were measured after carrying out manual dilution.

2.4. Procedure and treatment groups

20 male guinea pigs (Arlan, NL) were allocated for the study after authorization by the Animal Care and Use Committee (authorization IL-016-02-2008). The animals (average weight 388 ± 36 g) were housed in separate cages, and after 5 acclimatization days, were randomly divided into four groups. All animals were anesthetized by intramuscular injection of Ketamin (40–50 mg/kg) and Xylazine (4–5 mg/kg). The skin of the animals 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. [11]. Iron templates (circle, D = 40 mm) were immersed in water preheated to 75 °C and then placed in 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 1 × 10⁸ CFU/mL Pseudomonas aeruginosa using a micropipette. Each group was then treated with the relevant treatment option, as follows.

Group 1 was treated with a neutral non-adherent dressing material ([Melolin®], Smith & Nephew). Melolin® consists 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 dressing was placed directly on the burn, and was secured by an elastic adhesive bandage (Tensoplast™, Smith & Nephew).

Group 2 was treated with our composite dressing, derived from emulsion formulation I, which did not contain antibiotics. A round disc 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 3 and 4 were treated with the composite dressing derived from formulations II and III, respectively, which enabled fast and slow gentamicin release. The dressing materials were placed and secured as described in group 2. 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).

2.5. Post mortem examination

Animals were anesthetized after 10 and 14 days, and the dressing materials were removed. The closed (epithelialized) wound area and open (non-epithelialized, bleeding) wound area were traced on a transparent paper, and 5 mm biopsies were taken from the center of the wound and immediately fixed in phosphate-buffered formalin. The sections were stained with hematoxylin and eosin (H&E), and 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 (Table 1). The sections were evaluated based on structure and content. The sum of individual grades was then calculated to generate an overall grade between 0 and 24, with the maximal grade of 24 representing optimal restoration of skin. Means and standard error of the means were calculated for each data set (controls 1 and 2, fast and slow release). Differences between means were analyzed for statistical significance using one-way ANOVA with the Tukey-Kramer multiple comparisons post test (SPSS version 17.0). p values ≤0.05 were considered significant.

3. Results

3.1. Dressing structure

A composite wound dressing composed of a plain-woven polyglyconate mesh bound by a continuous poly-(DL-lactic-co-glycolic acid) (PDLGA) porous matrix loaded with gentamicin was developed and studied (Fig. 1a). The PDLGA matrix adhered well to the fibers, forming a skin layer with a

| Table 1 – Semi-quantitative grading of wound histology. |
|-------------|----------------|----------------|----------------|----------------|----------------|
| Scale | Epithelialization | Epidermis–dermis attachment | Collagen | PMNL + macrophage | Neo-angiogenesis | Adnexa |
| 0 | No regeneration | Absent | Absent | Absent | Absent | Absent |
| 1 | Little regeneration | Irregular | Mild | Moderate | Mild | Mild |
| 2 | Moderate regeneration | Prevalent | Moderate | Mild | Moderate | Moderate |
| 3 | Complete regeneration | Full | Marked | Absent | Marked | Marked |

PMNL – polymorphonuclear cells.
Fig. 1 – The structure of the biodegradable composite wound dressing composed of biodegradable fibers bound by a continuous porous PDLGA matrix. (a) Photograph of the wound dressing, (b) cross-sectional cryo-fractured SEM image demonstrating the plain-weave basic unit structure, (c) the microstructure of the porous matrix, (d) cumulative release of gentamicin from wound dressings derived from emulsions with 10% (w/polymer w) drug contents. (▲) Formulation II; 6:1 O:A phase ratio, stabilized with 1% (w/v) BSA. (■) Formulation III; 12:1 O:A phase ratio, stabilized with 1% (w/v) Span 80 (mean ± SEM).

Investigated dressing material

Fig. 2 – Representative photographs of wounds, ten and fourteen days after treatment with Melolin® (a1 and a2, respectively), a dressing material based on formulations devoid of gentamicin (control, b1 and b2, respectively), and with fast (c1 and c2, respectively) and slow (d1 and d2, respectively) release rates of gentamicin.
thickness of approximately 60 µm (Fig. 1b). The freeze-drying of inverted emulsions technique used to create the PDLGA porous matrix yielded a highly porous microstructure, shown in Fig. 1c, which also acts as a reservoir for the antibiotic drug incorporated in it.

3.2. In vitro drug-release studies

The cumulative release of antibiotics from dressings based on emulsion formulations containing 10% (w/w) gentamicin stabilized with BSA or Span are presented in Fig. 1d. The gentamicin release profile from the dressing stabilized with BSA typically demonstrated a relatively high burst release of antibiotics (68%), followed by a gradual release in a decreasing rate over time. The release from the formulation stabilized with Span demonstrated a considerably lower burst release (4%) and a longer overall release of gentamicin, with an almost constant release rate for 4 weeks.

3.3. In vivo evaluation

Overall, the animals tolerated the experimental procedure well and did not show signs of distress or suffer significant weight loss (> 10% of body weight). Two animals (10%) were lost within 24 h of burn infliction, possibly due to anaesthesia complication, and the remaining animals were sacrificed after ten or fourteen days.

The wound dressing materials remained in position over the course of treatment and were not disrupted. The dressing material created good contact with the skin, turning transparent in the exuding regions of the wound. All dressing materials used in the study were easily removed from the wound. Notable degradation of the binding matrix occurred in the regions subject to exudation, creating visible voids between the supporting fibers. This finding was supported by SEM photographs of different regions of the retrieved dressing material. The dressing’s margin demonstrated negligible degradation while its center demonstrated advanced degradation. The fibrous mesh remained intact despite degradation of the binding matrix.

3.3.1. Wound closure

Second degree burn wounds were evaluated macroscopically by two quantitative parameters ten and fourteen days after infliction of the burns: (i) percentage of the original area subjected to burn injury which was still an open wound, and (ii) wound contraction as depicted by the total wound area (epithelialized and non-epithelialized) as a percentage of the original area subjected to burn injury. Representative photographs of wounds treated with the various dressing materials and the two endpoints are presented in Fig. 2. Controlled release of gentamicin had a beneficial effect on wound closure. Ten days after the infliction of burns, an 88% of re-epithelization was observed with the fast release formulation and a 95% of re-epithelization with the slow release formulation (Fig. 3a). Despite a half-fold decrease in the open wound area compared to Melolin®, the superiority of the fast-release formulation was not proven statistically. However, the non-epithelialized area under the slow-release formulation was significantly smaller than with all other formulations (p ≤ 0.05), and 88% smaller than with Melolin®. All wounds were almost fully epithelialized two weeks after the infliction of burns.

3.3.2. Wound contraction

After ten days, fast and slow gentamicin-eluting dressing materials demonstrated less than 4% contraction compared to 17% and 26% contraction measured for the wounds treated with the dressing material devoid of antibiotics and Melolin®, respectively (Fig. 3b). After 14 days, wound contraction increased in wounds treated with the non-antibiotic-eluting materials (37% and 41%, respectively), while contraction in wounds treated with controlled release of gentamicin increased mildly to 15% and 14% for the fast and slow releasing formulations, respectively, which was significantly lower than with the non-antibiotic-eluting materials (p ≤ 0.05).

3.4. Histological wound evaluation

Histological sections of representative wounds taken from the four groups upon sacrifice at day 10 and day 14 are presented in Fig. 4. Scoring according to the semi-quantitative method described in Table 1 is presented in Fig. 5.

Ten days post-injury, wounds from both control groups were poorly epithelialized and demonstrated an enhanced inflammatory response (Fig. 4a and b). Hemorrhage was
present in both groups, and skin appendages were generally absent. Individual scores of the assessed parameters, especially collagen formation, inflammatory response and adnexa were slightly higher for wounds treated with the biodegradable dressing control compared to the Melolin control.

However, the general scores did not differ statistically (6.3 ± 1.9 and 3.0 ± 0.0 out of a maximum of 18, respectively, p = n.s.). Wounds treated with controlled release of gentamicin attained significantly higher histological scores than both controls after 10 days (12.8 ± 1.7 and 17.5 ± 0.3, for fast and slow release rates, respectively). This was mainly due to significant re-epithelialization of the wound. Wounds treated with a fast release rate of gentamicin were mostly re-epithelialized, while wounds treated with slow release of gentamicin were fully re-epithelialized with a well-structured epidermis layer. The granulation tissue that had occupied the

Fig. 4 – Histological section of guinea pig skin in Pseudomonas aeruginosa-contaminated deep second degree wounds after ten days treatment with: Melolin (a1), a magnified view demonstrates hemorrhage, destruction of the dermis and lack of epithelialization (a2); the investigated dressing material, devoid of antibiotics (b1), a magnified view demonstrates hemorrhage, and lack of epithelialization (b2); the investigated dressing material, with fast release of gentamicin (c1), a magnified view demonstrates good healing with very well organized epithelialization and dermis, without hemorrhage and leukocytosis (c2); the investigated dressing material, with slow release of gentamicin (d1), a magnified view demonstrates normal appearance of skin showing very well organized epithelialization and dermis as well as preserved hair follicles (d2).
base of the wound in the dermis was still rich in fibroblasts but contained few inflammatory cells. The presence of skin appendages was moderate to normal. Epithelialization was complete fourteen days post-injury in all four groups. Total scores of the controls (15.3/10 and 10.9/10 for Melolin® and the dressing devoid of antibiotics, respectively) were similar (p = n.s.) to those of the fast (13.7/10) and slow release (14.7/10) dressings.

4. Discussion

Previous experience has shown that uncomplicated burn wounds heal spontaneously within 3 weeks [20]. The endpoint of two weeks post-injury was chosen to correspond to the later stages of tissue formation and beginning of remodeling. At this stage, the wounds in all groups demonstrated advanced epithelialization. The two groups treated with controlled release of gentamicin were found to be only 10–13% non-epithelialized, while the controls were 23% non-epithelialized. Although results after fourteen days were generally very good compared to those reported previously in this model (75–100% non-epithelialized area at two weeks [20]), comparison between groups failed to indicate a significant improvement in epithelialization compared to Melolin®, for the biodegradable dressing material and for the additional controlled release of antibiotics. It is possible that some of the wounds were completely epithelialized before the fourteenth day, especially in the experimental groups, thus preventing evaluation of the healing rates between the different groups at this stage. However, significantly less wound contraction was measured for animals receiving controlled release of gentamicin (14–15%), compared to the controls (37–41%) and compared to previously reported assessments (40–43% [20]), indicating that the controlled release of gentamicin, whether at a fast or a slow rate, positively affects the healing process by enhancing the healing rate and thus slowing the contraction rate. Wound contraction is an ancient survival mechanism that allows animals to overcome injury and reduce the size of a wound without further treatment. However, it is an unfavorable process in humans, since it can lead to disfigurement of the skin and poor aesthetic results. It may also lead to loss of the normal flexibility of the skin—a fixed deformity that entails a functional disability, especially of the skin over the joints. Visible wound contraction is not usually evident until 5–9 days after injury, since significant fibroblast invasion into the wound area must occur before the onset of contraction [21]. Contraction is generally enhanced when the healing process is delayed. It is therefore advisable to cause wound closure as soon as possible. In practice, wounds which do not heal within 3 weeks are usually treated with skin grafts in order to decrease contraction and reduce the formation of hypertrophic scars, which are also increased upon delayed healing. Hypertrophic scarring does not occur in animals and therefore cannot be studied in an animal model.

Based on these findings it became clear that wound epithelialization and contraction need to be evaluated earlier in the healing process, to assess the progression of tissue formation and elucidate the effect of controlled release of gentamicin on the healing process. An additional set of animals was therefore sacrificed at an earlier endpoint of ten days. At this point, 43% and 35% of the wounds treated with Melolin® and with the dressing devoid of antibiotics remained non-epithelialized, whereas wounds treated with fast and slow controlled release of gentamicin underwent widespread epithelialization, demonstrating 22% and 5% non-epithelialized areas, respectively. It should be noted that wounds treated with fast release of gentamicin demonstrated epithelialization which is equivalent to that achieved after fourteen days of treatment with the conventional treatment and that even better epithelialization was achieved following slow release of gentamicin. This four-day fore in epithelialization represents a 40% reduction in healing time.

Despite a slightly higher contraction measured for the biodegradable material devoid of antibiotics, it performed similarly to Melolin® (p = n.s.) in epithelialization as well as contraction, thus indicating that the performance of the biodegradable dressing material itself is equivalent to Melolin®,
and does not evoke a negative effect due to its chemical, structural or physical properties. The findings also indicate that the controlled release of gentamicin from the wound dressing material significantly accelerates epithelialization (wound closure), thus reducing the effect of unfavorable contraction, which becomes a dominant mechanism when healing is delayed.

Evaluation of histological sections was performed in order to compare the structure and contents of the regenerating skin and facilitate statistical comparison between treatment groups. Histological sections of the wounds taken after fourteen days were fully epithelialized, with a normal skin appearance, and a well-organized epithelium and dermis. Averaged histological scores were between 10.9 (control 2) and 14.7 (Melolin) and did not differ statistically. These findings concur with the macroscopic findings, indicating that fourteen days represents a late stage in which tissue formation is close to complete whether it took an accelerated or natural course. Histological results after ten days demonstrate larger differences between the experimental and control groups. Specifically, better results were attained for the controlled release at a slow rate (17.5/18) compared to the fast rate (12.8/18). Wounds treated with Melolin® and the control devoid of antibiotics demonstrated a poor outcome (3.0/18 and 6.3/18, respectively). The most important factor was the absence of epithelium in these groups. Conversely, wounds treated with controlled release of gentamicin were mostly epithelialized and well-organized, indicating an advanced stage of healing. Furthermore, the quality of the epidermis–dermis junction was better. This finding implies good resistance to shearing (e.g., by minor trauma or removal of the dressing material which may strip away newly formed epithelium, causing bleeding and prolongation of the healing process). Another important finding in groups treated with controlled release of gentamicin was the maintenance of hair follicles and adnexa which are the source for re-epithelialization from within the burned area.

In conclusion, in vivo evaluation of the dressing material in a contaminated wound demonstrated its ability to accelerate wound healing compared to an unloaded format of the material and a non-adherent dressing material (Melolin®). Wound contraction was reduced significantly, and better quality scar tissue was formed. Faster epithelialization of the wound was measured in both release strategies, but was significantly better for animals treated with the slow release rate. From a practical aspect, faster epithelialization leads to less pain to the patient, shorter hospitalization, a better healing quality with less contraction and fewer hypertrophic scars. The gold standard local treatment with topical antibacterial agents, e.g., silverol®, requires daily or twice-daily replacements of the dressing material, which are time consuming and painful to the patient. Several of the dressing materials used today that provide controlled release of silver ions as an antibacterial agent have been shown to induce a toxic effect on cells, which can delay wound healing [5–8]. The current dressing material 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.

Conflict of interest

The authors state no conflict of interest.

Supporting information

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