Inflammatory inert poly(ethylene glycol)–protein wound dressing improves healing responses in partial- and full-thickness wounds

Kirill I Shingel, Liliana Di Stabile, Jean-Paul Marty, Marie-Pierre Faure

ABSTRACT

In this study, a novel soft hydrogel system based on the poly(ethylene glycol)–protein conjugates was evaluated as an occlusive wound dressing material. The hydrogel material, referred by the name of BioAquacare™, contains up to 96% of the liquid and is formulated with phosphate-buffered saline and safe preservative to control bacterial load in the open wounds. Performance of the BioAquacare™ as a wound dressing material was assessed in partial- and full-thickness wounds in pigs. Wound analysis comprised macroscopic determination of the wound size, histological examination of the healing tissues and biochemical characterisation of wound exudates. The wounds treated with BioAquacare™ healed without any signs of inflammation, skin irritation, oedema or erythema. Cellular composition of the reepithelialised wounds was very similar to that of the normal skin, with a well-developed stratum corneum and epithelial layer. It was observed that BioAquacare™ plays the role of a liquid compartment, which provides pronounced hydration effect and helps maintain a natural moist environment of the healing tissues. BioAquacare™ showed relatively low protein-absorbing activity, absorbing predominantly low-molecular-weight molecules, including interleukin (IL)-1β, IL-6, transforming growth factor-β1 and products of haemoglobin degradation. It is concluded that application of the moist BioAquacare™ dressing promotes fast reepithelialisation by creating favourable environment for keratinocytes proliferation and it also reduces scarring. The results show that BioAquacare™ can be considered as a safe, biocompatible and inflammatory inert wound dressing material.

Key words: Cytokine  Growth factor  Inflammation  Moist wound dressing  Poly(ethylene glycol)

INTRODUCTION

Biocompatibility of the wound dressing material is considered a necessary prerequisite for successful exploitation of the medical device because inherently initiated biological responses to foreign material affects the normal course of wound healing and may result in serious complications. The influence of the material chemistry of the dressings is especially important during the early stages of wound healing and is associated with the intensity and the duration of the inflammatory phase. Several studies have shown that the development of an inflammatory reaction from the wound against the dressing material plays a key role in wound reepithelialisation (1,2). In

Key Points

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BioAquacare\textsuperscript{TM}: an occlusive wound dressing material

In this study, we present in vitro and in vivo data showing the efficiency of the BioAquacare\textsuperscript{TM} formulation as a wound dressing material. Experimental models include partial- and full-thickness wounds in pigs. In this study, the efficiency of BioAquacare\textsuperscript{TM} in protecting the wounds and stimulating wound closure was compared with that of other existing products.

**Key Points**
- We have developed a wound dressing that acts as an occlusive, non-toxic medical device capable of protecting wounds from bacterial infection and providing an environment close to the physiological milieu of the extracellular matrix.
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- The experiments on the partial-thickness wounds were carried out in six young domestic pigs.
- Eight wounds were made in each experimental animal.
- Immediately after surgery, the BioAquacare\textsuperscript{TM} was applied to the wounds under occlusive conditions using Tegaderm\textsuperscript{TM} as a secondary dressing.
- Two animals were used for evaluation of BioAquacare\textsuperscript{TM}.

**MATERIALS AND METHODS**

**Preparation of BioAquacare\textsuperscript{TM}**

PEG–soy protein hydrogel was synthesised, and formulated aseptically in a class 100 clean room. An aqueous solution of activated PEG was mixed with an equal volume of the soy protein solution and the resultant mixture was cast between two films to form a hydrogel with a thickness of 1–8 mm. The hydrogel matrix reactivity appears because of the formation of the urethane links between free amino groups of the protein and PEG–carbonate moieties. After polymerisation, the hydrogel was incubated in a buffered solution to washout p-nitrophenol formed as a by-product of the cross-linking reaction. Finally, purified hydrogel was formulated with phosphate-buffered saline (PBS) containing ethylenediaminetetra-acetic acid and preservative at pH 5.5.

**Partial-thickness wounds**

The experiments on the partial-thickness wounds were carried out in six young domestic pigs weighing 15–18 kg. The animals were fed with a commercially grown diet and housed singly at 20–25\textdegree C. Experimental protocols were approved by the Ethical Committee of the Department of Veterinary Medicine at the University of Montreal. The animals were handled according to the ‘Guide for the Care and Use of Laboratory Animals’. The skin of the animals was shaved using a hair clipper and washed with a neutral soap. No aseptic solutions were applied on the skin. Four zones measuring 5 × 10 cm each were demarcated. Before surgery, the animals were anaesthetised with a mixture of azaperone and ketamine. General anaesthesia on surgery or wound dressing changes was maintained using isoflurane by mask. Eight, dorsal, 300-\textmu m thick wounds measuring 2 × 3 cm were made in each experimental animal using a Padgett dermatome. Immediately after surgery, the BioAquacare\textsuperscript{TM} was applied to the wounds under occlusive conditions using Tegaderm\textsuperscript{TM}. The wound dressings were changed daily until complete wound closure.
Key Points

- in the experiments on the full-thickness wounds, BioAquacare™ 2nd Skin®, Urgotul® and dry gauze were tested in 9 pigs
- the wound dressings were applied immediately after wounding and the animals were bandaged with Elastomer bandages
- the wound dressings were changed every 2 days after until complete wound closure
- the rate of wound closure was determined by the reduction in the wound size with the help of planimetry and digital photography
- the animals were euthanised at predetermined time-points and the dorsal skin of each pig was removed
- wound exudates were extracted from the dressings by incubating dressing materials
- wound analysis comprised microscopic determination of the wound size, histological examination of the healing tissues and biochemical characterisation of wound exudates

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Full-thickness wounds

In the experiments on the full-thickness wounds, BioAquacare™, 2nd Skin®, Urgotul® (Laboratoires Urgo, Chenôve, France) and dry gauze were tested in nine pigs using the guidelines and approvals described above. Before surgery, the skin of the animals was shaved and washed. General anaesthesia was performed as described above for partial-thickness wounds. Four full-thickness dorsal wounds reaching muscular fascia were made in each animal using a biopsy punch of 25 mm in diameter. The wound dressings were applied immediately after wounding, and the animals were bandaged with Elastomer bandages. The wound dressings were changed every 2 days until complete wound closure.

Macrosopic evaluation

The rate of wound closure was determined by the reduction in the wound size with the help of planimetry and digital photography. With every dressing change, the wound boundaries were traced using a template that repeated the size of the initial injury, and the changes in the wound size were recorded. Wound analysis also comprised microbiological determination of the wound infection, evaluation of the volume of granulation tissue and visual inspection for the signs of skin irritation, oedema, erythema and presence of scar. Additionally, each animal’s activity level and body growth was considered as a manifestation of animal’s comfort and well-being.

Histological examination

The animals were euthanised at predetermined time-points by an overdose of sodium pentobarbital. Immediately after sacrifice, the dorsal skin of each pig was removed and fixed in 10% neutral-buffered formalin. After 2 days, the fixed tissues were embedded in paraffin blocks. Wounded areas of the skin were identified and sliced at 5 μm. Haematoxylin and eosin staining was used for general observation, and Masson’s trichrome colouration was used for analysis of collagen organisation. The slides were analysed using a Leica DM4000B microscope equipped with a Leica DFC digital camera (Leica Microsystems AG, Wetzlar, Germany).

Biochemical characterisation of wound exudates

Wound exudates were extracted from the dressings by incubating dressing materials in PBS buffer at 4°C for 3 hours. In some cases, the extraction mixture media was sonicated for 20 seconds to facilitate protein dissolution. After 3 hours of extraction, aspirated liquid containing wound dressing extracts were centrifuged and the supernatants sterilely filtered. The supernatants were then frozen before further analysis. Wound protein extracts were prepared by washing the wound bed with 10 ml of sterile PBS for several minutes, as proposed by Saymen et al. (10). The samples of wound protein extracts were filtered sterilely and kept frozen. Total protein content was determined by bicinchoninic acid colorimetric assay using bovine serum albumin as a standard. Enzyme-linked immunosorbent assay (ELISA) kits for porcine interleukin (IL)-1β, IL-6 and transforming growth factor (TGF)-β1 were purchased from R&D Systems Inc. (Minnepolis, MN, USA) and used according to the manufacturer’s instructions. Immunoglobulin G (IgG) was detected by means of protein-A affinity chromatography on the HiTrap™ Protein A HP column from Amersham Biosciences (Uppsala, Sweden).

Component composition of proteins was also studied by means of reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC was performed in the gradient mode on a system consisting of a type 600E pump, a 776 autosampler and a 996 Photo Diode Array detector, all obtained from Waters (Milford, MA, USA). The column used was an Ace C8 (300 × 4.6 mm internal diameter (I.D.), particle size of 5 μm) silica-based column thermostabilised at 40°C. The mobile phase composed of 0.1% trifluoroacetic acid (TFA) in water (eluent system A) and 80% (v/v) acetonitrile in 0.075% TFA (eluent system B), which were pumped at a flow rate of 1.0 ml/minute, and the column effluent was measured within the wavelength range of 210–300 nm at a 4.8-nm resolution. The elution was carried out with a linear gradient of B from 0% to 90%
for 30 minutes, followed by the isocratic elution in 90% of B for 5 minutes.

**Statistical analysis**
Statistical analysis \( (n \geq 3) \) was performed on all quantitative data (wound size, total protein content, etc.) using an analysis of variance to a significance level of \( P \geq 0.01 \) or \( P \geq 0.05 \). The results shown in the tables and figures are the means and standard deviation. The content of the cytokines and growth factor is expressed in nanograms of the cytokine or the growth factors in 1 g of protein extracted from the dressing (ng/g).

**RESULTS**

**Partial-thickness wounds**
Reduction in the size of the partial-thickness wounds on application of BioAquacare\textsuperscript{TM} compared with that by the use of other products is shown in Figure 1A. Wounds treated with moist BioAquacare\textsuperscript{TM} dressings were completely reepithelialised after 6–7 days, whereas dry-gauze-covered wounds required a longer time to heal (Figure 1A). BioAquacare\textsuperscript{TM}-treated wounds showed no signs of infection or inflammations. This was in sharp contrasts with Tegaderm\textsuperscript{TM} treatment where large volumes of wound fluid trapped by the occlusive film led to scab formation (Figure 1B, day 6). A layer of scab had to be removed on every change of Tegaderm\textsuperscript{TM} dressing as this contamination precluded the accurate determination of wound size.

Low levels of inflammation in the BioAquacare\textsuperscript{TM}-treated wounds were confirmed by histological analysis of the tissue (Figure 2). The cellular composition of the wounds reepithelialised under moist occlusive conditions provided by BioAquacare\textsuperscript{TM} was very similar to that of the normal skin, with a well-developed stratum corneum and epithelial layer. Collagen was more or less completely remodelled to form a bundle texture. At the same time, moderate to severe inflammation was observed in the wounds treated with 2nd Skin\textsuperscript{TM} and Tegaderm\textsuperscript{TM} (Figure 2). These products induced intense host response monitored by an accumulation of mixed inflammatory infiltrates in a vicinity of newly formed epithelium. To a lesser extent, an inflammatory response was also observed in the dry-gauze-treated wounds (Figure 2).

**Full-thickness wounds**
All the wounds were closed 28–30 days after surgery, irrespective of the product used (Figure 3). In some instances, however, oedema, erythema and wound infection impeded the normal wound healing process. Oedema developed in the wounds covered

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**Key Points**
- Statistical analysis was performed on all quantitative data
- Wounds treated with BioAquacare\textsuperscript{TM} dressings were completely reepithelialised after 6–7 days and showed no signs of infection or inflammation while dry gauze covered wounds required a longer time to heal
- A layer of scab had to be removed at every Tegaderm\textsuperscript{TM} dressing and this contamination precluded the accurate determination of wound size
- In the BioAquacare\textsuperscript{TM} treated wounds, low levels of inflammation were confirmed, under the moist occlusive conditions, the cellular composition of wounds reepithelialised which was very similar to normal skin
- Collagen was more or less completely remodelled to form a bundle texture
- Moderate to severe inflammation was observed in the wounds treated with 2nd Skin\textsuperscript{TM} and Tegaderm\textsuperscript{TM}. An inflammatory response was also seen in the dry gauze treated wounds

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![Figure 1](image-url)  
**Figure 1.** (A) Reepithelialisation of the partial-thickness wounds in pigs and (B) appearance of the wounded sites after removal of the dressings. Compared with the dry gauze, both BioAquacare\textsuperscript{TM} and Tegaderm\textsuperscript{TM} provoke faster wound closure, but wounded sites appeared differently. Wounds treated with Tegaderm\textsuperscript{TM} were covered with a scab (seen as a black area in the right bottom of the picture), as opposed to clean wounds dressed with BioAquacare\textsuperscript{TM}.
with 2nd Skin® resulted in a significant expansion of the wounds at the beginning of the repair period. Also, occasional reopening of these injuries because of bacterial infection was detected at the later stages of wound healing, as seen by the increases in wound areas shown on days 15–18 in Figure 3A.

Histological examination of the wounds showed noticeable differences in response to the injuries and/or wound dressing application (Figure 4). After 10 days of treatment, the wounds treated with BioAquacare™ were completely filled with granulation tissue and populated with fibroblasts (Figure 4A). Newly synthesised collagen fibers, albeit not fully remodelled, were deposited in a basket-weave pattern, surprisingly similar to that of intact dermis. At day 10, the wounds were reepithelialised by 75–80%, and neoepidermis was found to be well stratified and keratinised...

**Key Points**
- all wounds were closed 28-30 days after surgery irrespective of the product used
- in some cases however, oedema, erythema and wound infection impeded the normal wound healing process
- occasional reopening of the injuries because of bacterial infection was detected at the later stages of wound healing
Healing of the BioAquacare™-dressed injuries proceeded with minimal inflammation, as was judged from the relatively small accumulation of inflammatory cells in the area of injury. This contrasts with the response in the gauze-covered wounds, where manifestations of severe inflammation were detected in the subcutaneous and perivascular tissues and also in the periphery of the injuries (Figure 4B). Starting from day 10, acute inflammation began to subside, but complete resolution of inflammation was observed only in the 20-day-old wounds. After 3 weeks, immature cell-dense granulation tissue with poorly organised collagen deposits was still present in the upper dermal layer.

**Interactions with exudates**

Serum albumin, fibrinogen, fibrin monomers and IgG were identified as the most abundant components of the wound dressing extracts (Figure 5). The results of quantification of serum albumin and fibrinogen from high-performance liquid chromatography data are shown in Table 1 together with the data on the IgG determination. The amount of the assayed proteins decreases gradually as the partial-thickness wounds heal. However, initial concentration of the different protein varied greatly in the different types of dressings. The most efficient absorption of fibrinogen, IgG and serum albumin is observed with dry bondage dressing. The content of these proteins in the hydrogel materials was 30–80 times less, indicating that hydrogel dressings were less effective in absorbing relatively high-molecular-weight proteins.

A pronounced wound cleansing effect of BioAquacare™ was monitored in the experiment on partial-thickness wounds by the changes in hydrogel colour. An initial colourless transparent material of BioAquacare™ hydrogel changed to yellow or brown after application to the 6 day wounds.

- The ability to efficiently eliminate products of cell degradation from healing wounds is not a common attribute to wound dressing products.
- BioAquacare™ dressing appeared to be more absorbent for IL-1β compared with dry gauze.
- Moist occlusive conditions promote an inverse relation between IL-1β and TGF-β1 levels.

**Key Points**

- Healing of the BioAquacare™-dressed injuries proceeded with minimal inflammation.
- This contrasts with the response in the gauze-covered wounds, where manifestations of severe inflammation were detected in the subcutaneous and perivascular tissues and also in the periphery of the injuries.
- Hydrogel dressings were less effective in absorbing relatively high molecular weight proteins.
- A pronounced wound cleansing effect of BioAquacare™ was monitored in the experiment on partial-thickness wounds by the changes in hydrogel colour.
- An initial colourless transparent material of BioAquacare™ hydrogel changed to yellow or brown after application to the 6 day wounds.
- The ability to efficiently eliminate products of cell degradation from healing wounds is not a common attribute to wound dressing products.
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- Moist occlusive conditions promote an inverse relation between IL-1β and TGF-β1 levels.
BioAquacare™ is biocompatible with other dressings and reepithelialisation expected because of the presence of low-molecular-weight products of haemoglobin degradation, as compared with inflammatory inert, as designed PEG–protein hydrogel matrix of BioAquacare™ appeared to be a unique feature of the PEG–protein material. Indeed, such an ability of the material to efficiently eliminate products of cell degradation from healing wounds is not commonly attributed to the wound dressing products.

Analysis of the wound dressing extracts recovered from full-thickness wounds included quantification of IL-1β, IL-6 and TGF-β1 as biochemical markers in the different stages of the wound healing process. The concentration of these molecules determined by ELISA was then normalised to the total protein content in a particular sample of the wound fluid extract. The results of such an analysis for IL-1β and IL-6 are summarised in Figure 7.

A rapid increase of the IL-1β content is observed at day 2, irrespective of the wound dressing used (Figure 7A). This increase is symptomatic for the preliminary inflammatory response to the injury. Starting from day 6, BioAquacare™ dressing was found to continuously absorb IL-1β from the wound site until the end of monitoring at day 14. During the period of 6–14 days, the fraction of this cytokine extracted from BioAquacare™ was found to be two to three times higher compared with that from both 2nd Skin® and dry gauze (Figure 7A). Similar phenomena were observed when the content of IL-6 was studied (Figure 7B). In view of the relatively low

Table 1: Acute-phase proteins in the wound exudates recovered from different dressings after application to the partial-thickness wounds in pigs

<table>
<thead>
<tr>
<th>Protein*</th>
<th>Product</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (Mr = 340 kDa)</td>
<td>BioAquacare™</td>
<td>7-1 (1-2)</td>
<td>10-2 (2-4)</td>
<td>10-3 (1-8)</td>
<td>4-9 (2-2)</td>
</tr>
<tr>
<td></td>
<td>2nd Skin®</td>
<td>25-9 (3-8)</td>
<td>51-7 (12-2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Dry gauze</td>
<td>573-0 (88-1)</td>
<td>63-9 (17-3)</td>
<td>64-8 (3-5)</td>
<td>29-6 (1-8)</td>
</tr>
<tr>
<td>IgG (Mr = 170 kDa)</td>
<td>BioAquacare™</td>
<td>25-2 (18-1)</td>
<td>14-9 (8-2)</td>
<td>11-9 (0-4)</td>
<td>11-3 (2-3)</td>
</tr>
<tr>
<td></td>
<td>2nd Skin®</td>
<td>72.7 (19-1)</td>
<td>52.6 (9-5)</td>
<td>18-3 (7-1)</td>
<td>8-5 (0-4)</td>
</tr>
<tr>
<td></td>
<td>Dry gauze</td>
<td>1133-3 (189-0)</td>
<td>228-9 (58-1)</td>
<td>193-1 (144-1)</td>
<td>136-4 (71-1)</td>
</tr>
<tr>
<td>Serum albumin (Mr = 66 kDa)</td>
<td>BioAquacare™</td>
<td>56-4 (12-7)</td>
<td>81-9 (24-2)</td>
<td>97-3 (17-4)</td>
<td>36-4 (13-4)</td>
</tr>
<tr>
<td></td>
<td>2nd Skin®</td>
<td>159-9 (82-1)</td>
<td>183-4 (17-9)</td>
<td>81-9 (20-5)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Dry gauze</td>
<td>1674-7 (231-0)</td>
<td>360-3 (112-8)</td>
<td>216-0 (54-3)</td>
<td>12-1 (7-9)</td>
</tr>
</tbody>
</table>

*Content of the proteins is expressed in micrograms of protein per gram of the dressings (μg/g). The data (n = 3) are given as mean (SD).
level of the total proteins in the BioAquacare™, an increased amount of IL-1β and IL-6 recovered from this material (Figure 7) can be interpreted as a manifestation of the selective absorption of the low-molecular-weight proteins by the hydrophilic matrix of BioAquacare™. Confirmation of this hypothesis was obtained when residual concentration of IL-1β in wound beds was determined and compared with the concentration of this cytokine recovered from the dressings, as shown in Figure 8. Indeed, BioAquacare™ dressing appeared to be more absorbent for IL-1β compared with dry gauze. As shown in Figure 8, the absorption of the wound fluid by dry bondage results in almost complete removal of the available proteins from wound bed, while the concentration of IL-1β in the wound remains high.

No significant amount of TGF-β1 was detected in the BioAquacare™ samples 4 days after wounding, albeit other types of dressing products contained traceable amounts of TGF-β1. Similarity in IL-1β and TGF-β1 levels in the period of 4–12 days postwounding prompted us to perform a more detailed analysis. Results of the TGF-β1 content determination were plotted against the amount of IL-1β, as shown in Figure 9. Surprisingly, all data points were found to be grouped into two categories symptomatic for either dry or moist wound healing situation. As shown in Figure 9, the relative low level of IL-1β associated with elevated secretion of TGF-β1 is characteristic for dry wound healing, while moist occlusive conditions promote an inverse relationship between IL-1β and TGF-β1 levels.

DISCUSSION

Complete recovery of skin integrity and function is an ultimate goal of wound care practices. Despite recent advances in understanding wound biology and availability of modern wound dressing products, skin repair defects because of wound infection and/or...
excessive scarring are still a serious concern. In this study, we tested new PEG–protein hydrogel as a wound dressing material in the porcine model of partial- and full-thickness wounds, which were chosen as a good representation of typical superficial wounds, skin lacerations, burns, skin grafts, etc. According to the recent analysis by Sullivan et al., the porcine model is an excellent tool to study wound healing in humans (14).

The efficiency of the newly designed PEG–protein hydrogel in stimulating wound repair and reepithelialisation exceeded all our expectations. Although the rate of reepithelialisation of the Tegaderm™-, 2nd Skin®- and BioAquacare™-dressed wounds was comparable, the appearance of the wounds was different both macroscopically and histologically (Figures 1B and 2). BioAquacare™ is biocompatible and inflammatory inert, as compared with inflammatory responses in the wounds treated with other dressings (Figures 2 and 4).

In contrast to the dry gauze dressing, BioAquacare™ hydrogel showed relatively low protein-absorbing activity, interacting predominantly with low-molecular-weight species, including IL-1β, IL-6, etc. (Figure 8). In vitro studies on the mechanism of hydrogel–solute interactions showed that a primary contact of the protein-rich wound fluid with the hydrogel surface leads to dissolution of the proteins, followed by redistribution of the biopolymers in the hydrogel matrix (R. Snyders, O. Zabeida, K. I. Shingel, C. Roberge, M. P. Faure, L. Martinu, J. E. Klemberg-Sapieha, Ecole Polytechnique, Montreal, personal communication). As it is expected from the viewpoint of

Figure 8. Distribution of IL-1β (A) and acute-phase proteins (B) between wound bed and dressing materials after application on the 4-day-old full-thickness wounds. IL, interleukin.

Figure 9. Content of IL-1β plotted against corresponding content of TGF-β1 in the wound dressings taken from 4-to 14-day-old wounds. •, dry gauze; ○, BioAquacare™; △, 2nd Skin®. IL, interleukin; TGF, transforming growth factor.
the hydrogel structure, smaller proteins migrate faster than larger ones towards the hydrogel, resulting in the selective absorption of the relatively low-molecular-weight species, i.e. IL-1β, IL-6 under in vivo conditions.

Likely, selective absorption of IL-1β and IL-6 by BioAquacare™ has a positive effect on wound repair. For example, complete elimination of IL-1β from the wound site was shown to impair wound closure (15), whereas overexpression of this cytokine may prolong an inflammatory response, leading to the appearance of poorly healing injuries (16). An increased level of IL-1β was reported to be characteristic of the difficult non healing wounds, such as a leg ulcer (17), where the remodelling phase of the healing is retarded because of continuous inflammation.

Although several lines of evidence showed that IL-6-deficient animals display impaired wound healing because of inefficient inflammation, granulation tissue formation and reepithelialisation (16,18), an excess of IL-6 is symptomatic for poorly healing wounds (17) and has been regarded as a primary cause of scarring (19,20). Our data showed that the moist BioAquacare™ dressing contains comparatively higher amount of IL-6 than the dry bondage dressing (Figure 7), which might be interpreted as a manifestation of the intense inflammatory reactions in hydrogel-treated injuries. Such interpretation, however, would contradict to the results of wound analysis (Figure 4) and recent reports showing that an increased level of IL-6 (and also a decreased level of TGF-β1) is symptomatic for the neutrophil-depleted wounds with minimal infiltration of inflammatory cells (21). It was recently shown by Mori et al. (22) that reduced cell activation and as a consequence, attenuated infiltration of inflammatory cells under the condition of the limited cytokine signalling is not necessarily associated with impaired cutaneous repair. The latter observation finds additional confirmation in the present study.

A re-increase of the IL-1β content in 6- to 8-day-old wounds (Figure 7) is interpreted to be mainly as a result of activity of the proliferating keratinocytes (23). Additional evidence for this conclusion is now found from the comparison of the IL-1β and TGF-β1 contents (Figure 9). The level of TGF-β1 as a profibrotic growth factor with a well-documented inhibitory effect on keratinocyte proliferation is found to be relatively low for healing under moist conditions. This might indicate that the application of the moist dressing at the later stages of wound closure stimulates keratinocytes to synthesise chemotactic IL-1β and suppresses production of TGF-β1.

In summary, the data in this study show the efficiency of the PEG–protein hydrogel material as an inflammatory inert wound dressing material. It is observed that BioAquacare™ plays the role of a liquid compartment, which provides a pronounced hydration effect and helps maintain a natural moist environment of the healing tissues. BioAquacare™ showed relatively low protein-absorbing activity, interacting predominantly with low-molecular-weight species, including cytokines, growth factors and product of haemoglobin degradation. The study of the interactions between BioAquacare™ and cytokines suggests that the hydrogel matrix of BioAquacare™ is essentially suitable for the integration of cytokines and growth factors, thus creating an opportunity for the development of the biologically active wound dressing device.

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