Two Hydrocolloid Dressings Evaluated in Experimental Full-thickness Wounds in the Skin

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Hydrocolloid occlusive dressings are beneficial in wound management in many respects, although the adhesive matrix may disintegrate when in contact with wounds. The purpose of this study was to determine: (1) if material from two hydrocolloid dressings – Comfeel and Duoderm – showing differences in adhesive cohesion, can be chemically identified in granulation tissue; and (2) if the presence of this material influences cutaneous wound healing.

In full-thickness skin wounds in rats, components from the two hydrocolloid dressings were phagocytosed as indicated by the presence of foam cells. Extracellular vacuoles (100–400 μm in size) occupied about 25% of the granulation tissue volume in the Duoderm group but less than 5% in the Comfeel group, a statistically significant difference ($p < 0.001$). The vacuoles contained hydrophobic polymers derived from the respective hydrocolloid dressing, as analyzed by Fourier Transform Infrared (FT-IR) microscopy. Wound contraction did not differ significantly between the two hydrocolloid dressings. Wounds treated with Comfeel were significantly ($p < 0.05$) more epithelialized (mean: 78%) than those treated with Duoderm (mean: 41%). The proliferative activity in wound epithelium, as measured immunohistochemically by bromodeoxyuridine incorporation, was similar for the two treatment groups, indicating that epithelial migration was impaired in Duoderm-treated wounds.

In summary, extensive incorporation of hydrophobic dressing material from hydrocolloid dressings may render the wound bed less suitable for epithelial migration during acute secondary wound healing. *Key words: occlusive dressings; wound contraction; epithelialization; FT-IR microscopy.*

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INTRODUCTION

Occlusive wound treatment is becoming more widespread. Many different brands of occlusive dressings are available today; the main types are non-absorptive polymeric film dressings or absorptive hydrocolloid dressings (1–4). Previously we have reported enhanced healing of standardized full-thickness excisions in the skin of rats with a hydrocolloid occlusive dressing (Duoderm) compared with conventional wet-to-dry gauze treatment (5). It was further shown that foreign material was phagocytosed by macrophages resulting in foam cells, i.e. morphologically altered macrophage and extracellular vacuoles (5). The total composite dressing, i.e. the hydrophobic matrix and the hydrophilic particles, was responsible for the appearance of this altered macrophage

rather than a specific component of the hydrocolloid dressing (6). Comparative studies in pigs indicated histological differences in tissue response, depending on the composition of the hydrocolloid dressing (7–9). It was concluded from these studies that the integrity of the adhesive matrix was decisive for the amount of deposition of foreign material into the granulation tissue. However, neither the chemical composition nor the influence on wound healing of the deposited material was elucidated in these studies.

This investigation was carried out to compare Duoderm with another hydrocolloid dressing, which is more resilient to disintegration when in contact with wounds – Comfeel ulcer dressing – in the treatment of full-thickness wounds in rats regarding degree of tissue response to and identification of dressing material in the wounds, and healing (wound contraction and epithelialization). We used Fourier Transform Infrared (FT-IR) microscopy to determine the chemical composition of deposited dressing material in situ. Furthermore, proliferation in the wound epithelium was studied after labelling in vivo with the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU.)

MATERIALS AND METHODS

Animals

Fourteen Sprague-Dawley male rats, weighing 350–550 g, were used. The animals were fed a basal diet ad libitum and housed individually in our animal facilities at controlled temperature (19–21°C) and light (12 h light/12 h dark). The study protocol was approved by the University of Miami Animal Use Committee and the local ethics committee of the University of Linköping.

Surgical procedures and dressing of wounds

The rats were anaesthetized by one intramuscular injection (0.5 ml) of a mixture of ketamine hydrochloride (100 mg/kg) and xylazine (5 mg/kg). The hair was removed with electric clippers and the skin cleansed with 70% ethanol. Two uniform circular full-thickness wounds (each 3.34 ± 0.38 cm^2, mean $\pm$ SD, $n = 28$) were made in the thoracic region (one wound on each side of the spine), using a sterile 20-mm punch with the skin double-folded. After hemostasis and complete wound retraction the wounds were traced on a transparent plastic film for area determination ($A_{Day\ 0}$). The wounds ($n = 24$) were treated either with Duoderm (ConvaTec, New Jersey, USA; the original formulation of Duoderm was used in the present study, a formulation which has been supplemented with a more resilient type named Duoderm CGF) or Comfeel ulcer dressing (Coloplast A/S, Denmark). These two hydrocolloid dressings differ mainly with respect to the binder system and cohesion of the adhesive matrix. The adhesive of Comfeel is composed of styrene-isoprene-styrene co-polymer and sodium carboxymethyl cellulose (NaCMC), and that of Duoderm of polyisobutylene, NaCMC, pectin and gelatin. The presence of pectin in Duoderm results in acidic pH. In every other animal the left wound was treated with Duoderm and the right wound with Comfeel and vice versa. In two of the rats both wounds ($n = 4$) were treated with an adhesive polyurethane film (OpSite, Smith & Nephew, UK), which

served as occlusive non-hydrocolloid control in the histological studies (6). Dressings were applied to the wounds, extending over at least 3 cm of surrounding uninjured skin. Gauze swabs were placed over the dressings, and finally an elastic adhesive bandage (Elastoplast, Beiersdorf) was wrapped twice around the trunk of the animals to protect the dressings. The animals were checked daily for any dressing displacement and/or for leakage.

Analyses on post-operative day 10

Wound contraction. The rats were euthanized with an overdose of xylazine (1 ml). Five animals treated with hydrocolloid dressings were given BrdU (Sigma; 10 mg/ml 0.9% NaCl) intraperitoneally (50 mg/kg) 1 h before euthanization. The outer margins of the wounds were traced on a transparent plastic film for area determination ($A_{Day\ 10}$). The tracings were performed using computerized digital planimetry (10). Wound contraction was calculated as: $(A_{Day\ 0} - A_{Day\ 10})/A_{Day\ 0}) \times 100\%$.

Histology. Wounds were excised perpendicularly to the long axis of the elliptically shaped wounds and fixed in 4% buffered formaldehyde. The fixed wounds were embedded in paraffin and 5-μm thick sections were cut from each paraffin block. The sections from the middle of the wounds were stained with hematoxylin and eosin and examined in a blinded fashion using light microscopy. The epithelial coverage, i.e. wound surface length covered with at least one epithelial cell layer, was measured in the sections and given as total epithelium length coverage in percentage of the total wound surface. Epithelial proliferation was measured as BrdU incorporation and detected immunohistochemically using the protocol described by Nylander et al. (11) and an assay kit from Boehringer Mannheim (Cat. No.: 1 299 964). Briefly, after dewaxing and rehydration, the sections were digested with 0.4% pepsin in 0.01 M HCl at 37°C for 30 min. After a PBS-rinse the sections were treated with 2 M HCl at room temperature for 25 min and incubated with mouse monoclonal anti-BrdU for 18 h at 4°C. Incubation with alkaline phosphatase-conjugated secondary antibody was carried out for 30 min at 37°C, and a BCIP/NBT substrate system (Boehringer Mannheim) was used for visualization. The slides were counterstained with Mayer's hematoxylin for 2 min and mounted. Proliferation was quantified by light microscopy by manual counts of BrdU-positive stained epithelial cells per mm length of epithelium at a total magnification of 400.

The cross-sectional area of granulation tissue and that of extracellular vacuoles was estimated morphometrically, viewing slides in a microscope interfaced with a video camera and a monitor, and a personal computer using a special morphometric software.

FT-IR characterization of the extracellular vacuoles. To identify the material in the extracellular vacuoles, FT-IR microscopy was used on two Comfeel and two Duoderm-treated wounds in two of the rats. The FT-IR microscopy technique enables reliable chemical analysis of spots with diameters from 10 μm upwards. 5-μm sections of snap-frozen wound tissue in Tissue-Tek® were cut in the direction from the subcutaneous part toward the wound surface in a cryomicrotome at −20°C and placed on for infrared light transparent 1–1.5-mm thick KBr tablets of optical grade. A Perkin-Elmer FT-IR system 2000 microscope with a nitrogen-cooled narrow-band mercury-cadmium-telluride detector was used. For each transmission spectrum 32 scans were co-added at 4 cm^{-1} resolution in the mid-IR range of 4,000–700 cm^{-1}. A 100-μm circular aperture was used for Duoderm-treated wounds and a 50-μm aperture for Comfeel-treated wounds. Reference spectra were obtained of the hydrophobic binders of the respective dressing.

Statistics

Statistical evaluations were made by the paired Student's t-test. A probability of less than 0.05 was accepted as significant. Numeric data are presented as mean ± SD (standard deviation).

RESULTS

Macroscopic observations

The dressings remained in place during the entire experimental period. The adhesive of the Duoderm dressing dissolved and formed a gelatinous mass on the wound surface. In contrast the adhesive of the Comfeel dressing remained macroscopically intact.

Histological features

Foam cells were seen in the granulation tissue from wounds treated with both hydrocolloid dressings but not in OpSite-treated wounds. A conspicuous finding in the Duoderm group was the abundance of extracellular vacuoles in the granulation tissue, occupying 23.3 ± 14.3% (mean ± SD) of the granulation tissue volume. These contained condensed spherical eosinophilic material (Fig. 1a). Though present in Comfeel-treated wounds as well, extracellular vacuoles were significantly ($p < 0.001$) less common (4.6 ± 4.5%) and smaller in size (Fig. 1b). No extracellular vacuoles were seen in OpSite-treated wounds.
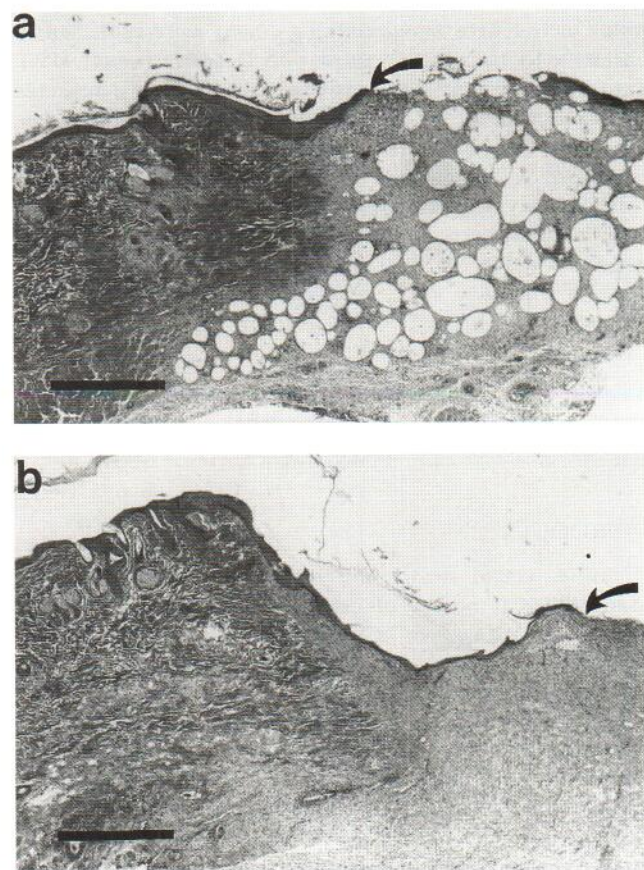

Fig. 1. Cross-section of full-thickness wounds treated with Duoderm (*a*) or Comfeel (*b*) hydrocolloid dressings on post-operative day 10 stained with hematoxylin and eosin. *Arrows* indicate the edge of migrating epithelium. Note abundance of extracellular vacuoles in Duoderm-treated wounds. *Scale bar* = 500 μm.

FT-IR investigations of the extracellular vacuoles

The extracellular vacuoles were easily identified and seen as droplets in cryosections of the wounds due to their amorphous appearance. The droplets were larger in Duoderm- (Fig. 2a) than in Comfeel-treated wounds at the same level as the histological findings (Fig. 2b). The spectrum shown in Fig. 2c of a Duoderm-treated wound is the average obtained from scanning the central circular (100 μm) part of the droplet 32 times. This spectrum is essentially identical to that obtained of the hydrophobic part (polyisobutylene) of the Duoderm hydrocolloid dressing (Fig. 2c, insert) and displayed no resemblance to the spectrum of adjacent granulation tissue. The broad peaks at 3,300, 1,654 and 1,541 cm^{-1} indicate the presence of several secondary amides as in polypeptides. The FT-IR spectrum generated from Comfeel droplets (Fig. 2d) was identified as the hydrophobic part of the Comfeel dressing (Fig. 2d, insert), with some interference from polypeptides from the wound. The more pronounced interference by polypeptides was most likely due to the small size of the extracellular vacuoles in Comfeel-treated wounds despite the use of a 50-μm aperture. The results were reproducible for both treatment groups.

Wound healing

The two hydrocolloid dressings did not differ in their effect on wound contraction. The mean epithelial coverage, as measured microscopically and blindly, was significantly higher with the Comfeel dressing than with the Duoderm dressing (Table I). Five of the 12 Comfeel-treated wounds were completely epithelialized compared with 2 of the 12 Duoderm-treated wounds.

To elucidate whether the difference in epithelial coverage was due to differences in migration or proliferation or both, we studied epithelial proliferation in the wounds by BrdU incorporation. BrdU staining was localized to the cell nucleus. In the migrating epithelium few keratinocytes stained at the
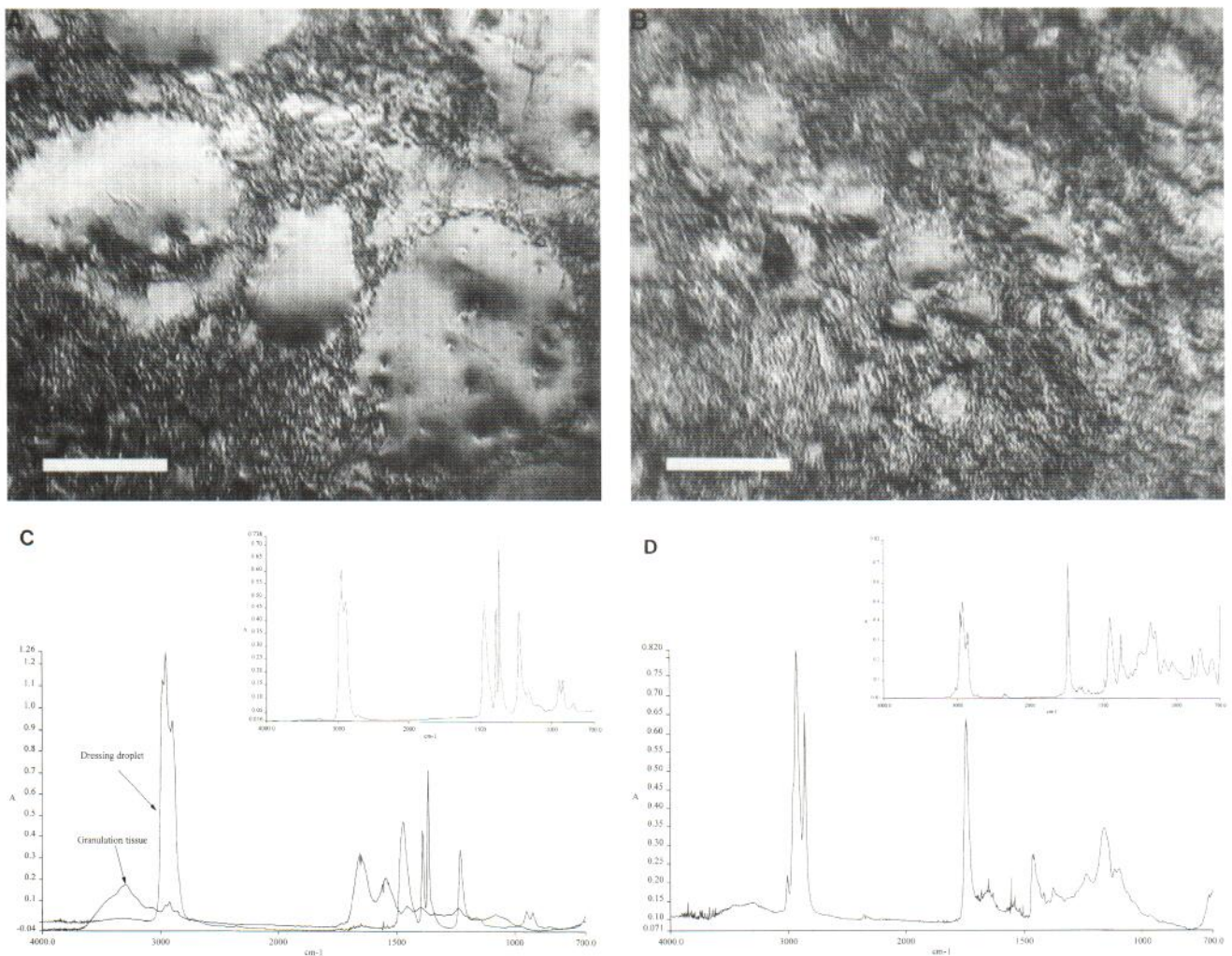

Fig. 2. Unstained cryosections of wounds treated with Duoderm (*a*) or Comfeel (*b*), as viewed through the FT-IR microscope. The droplets of dressing residues in the wound tissue are larger in the Duoderm-treated wounds than in the Comfeel-treated wounds. *Scale bar* = 100 μm.

The corresponding spectra of the droplets in Duoderm and Comfeel-treated wounds are shown in *c* and *d*, respectively. A spectrum of adjacent granulation tissue without visible droplets is superimposed on that obtained of a dressing droplet in (*c*). Reference spectra of respective hydrophobic matrix components for the two hydrocolloid dressings are shown as *inserts* in *c* (polyisobutylene; Vistanex LMMH, Exxon) and *d* (styrene-isoprene-styrene co-polymer (Cariflex, Shell), resin and plasticizer).

Table I. *Effect of hydrocolloid dressing on wound contraction and epithelialization of full-thickness wounds on post-operative day 10*

	Duoderm ($n = 12$)	Comfeel ($n = 12$)
Wound contraction (%)	81.0 ± 9.1	84.1 ± 7.6
Epithelialization (%)	41.3 ± 27.3	77.6 ± 23.1*

n = number of wounds assessed.
Mean $\pm$ SD.
* $p < 0.05$ compared with Duoderm.

epithelial tongue, whereas keratinocyte proliferation peaked about 1 mm distally from the epithelial tongue. There was no statistically significant difference in the number of BrdU-positive cells in wound epithelium between wounds treated with Comfeel (22.2 ± 12.5 positive BrdU cells/mm epithelium) or Duoderm (21.9 ± 13.1). The corresponding value in adjacent uninjured skin was 10.6 ± 0.8 positive BrdU basal cells/mm epithelium. In the granulation tissue endothelial cells showed high proliferative activity compared with fibroblastic cells.

DISCUSSION

The beneficial effects of hydrocolloid dressings are well documented. This group of wound management products provide an occlusive wound environment conducive to epithelialization. They also have the advantage of absorption of wound fluid, reducing the number of dressing changes. However, the absorption process is associated with disintegration of the adhesive matrix to varying degrees. Disintegration of the adhesive can lead to unwanted deposition of non-biodegradable dressing components in the wound. We have demonstrated differences between two commonly used hydrocolloid dressings. The hydrocolloid dressing with low adhesive matrix cohesion (Duoderm) deposited significantly more dressing material in the wound than the other, with high cohesive strength (Comfeel). Further, the extensive incorporation of dressing material resulted in impaired wound healing, specifically epithelial migration.

Mechanistically, our findings shed light on wound closure mechanisms. Interestingly, the abundance of foreign hydrophobic material in granulation tissue did not appear to influence wound contraction. The recent study by Gross et al. (12) indicated that the contractile forces are *not* generated within the granulation tissue in wounds in immobile skin type, confirming their previous findings in loose skin species (13). Our findings seem to support their conclusions because one would expect that less force would be generated in granulation tissue devoid of 25% of contractile elements, as was the case in Duoderm-treated wounds.

The mechanism of decreased epithelialization in Duoderm-treated wounds was delineated by BrdU-labeling in vivo. There appeared to be no negative effect on epithelial proliferation by the abundance of synthetic hydrophobic matrix components in the wound tissue, indicating that no toxic soluble factors were released from the incorporated material. Therefore, the reduced epithelialization could rather be explained by an impaired migratory ability. Woodley et al. (14) studied the effect of different substrates on keratinocyte migration in vitro. They found that a surface of polystyrene was a poor substrate in comparison with the same surface coated with collagen,

experimental conditions that mimic the contact of keratinocytes with wound surface with or without incorporated foreign hydrophobic polymeric material. It is thus conceivable that hydrophobic polymers (polyisobutylene) at the wound surface (Fig. 1a) can also inhibit epithelial migration in vivo.

Since the two hydrocolloid dressings also differ regarding the hydrophilic components, it cannot be excluded that the effect on epithelial migration was due to these differences. In another study, however, sodium carboxymethyl cellulose, gelatin and pectin did not influence wound repair measured as breaking strength when introduced into incisional wounds in the rat (6).

Further, our findings indicate there is room for improvements in the formulation of the adhesive matrix of these types of wound dressings. The formulation of Duoderm has been changed after the completion of this study. The improved formulation has increased cohesive strength, which results in significantly less deposition of dressing materials in wounds (9).

Phillips et al. (15) were unable to demonstrate the histological differences in chronic wounds we have shown here in fresh surgical wounds in the rat. Other investigators have, however, reported similar histological abnormalities in acute human wounds as we have shown in acute experimental wounds (8, 16).

In conclusion, extensive deposition of synthetic hydrophobic polymers derived from dressings can influence wound healing adversely. Hydrocolloid wound dressings with low cohesive strength should be replaced by dressings resilient to disintegration when in contact with wounds.

ACKNOWLEDGEMENT

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