

An Amorphous Hydrogel Enhances Epithelialisation of Wounds*

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Hydrogel dressings are gaining increased clinical acceptance as a wound management modality. The purpose of this study was to compare the effect of three amorphous hydrogels with occlusive, control treatment (Tegaderm®) on healing of experimental wounds.

Eight partial-thickness cutaneous wounds (2.5 cm × 2.5 cm × 0.04 cm) were made, using an electrokeratome, and the four treatments were allocated by randomisation within a cephalad and a caudal region on each of six 60-kg domestic pigs. In total, twelve wounds were each treated with 2.0 ml of each type of hydrogel – an experimental amorphous hydrogel (“Exgel”), IntraSite® Gel and a poloxamer gel containing 3% hydrogen peroxide – and covered with Tegaderm®, or treated with Tegaderm® alone. At 66 h post-operatively, formalin-fixed, paraffin-embedded sections of wounds were hematoxylin-eosin-stained and assessed morphometrically for epithelium coverage in a blinded fashion.

The Exgel remained macroscopically intact on the wounds in contrast to the other hydrogels, which had dissolved completely after treatment. Exgel significantly ($p < 0.05$) increased epithelial coverage of the wounds, compared with the other treatments (by 20% more than Tegaderm®-treated wounds). In vitro experiments indicated that the polymeric matrix of Exgel sequesters bioactive molecules present in wound fluid and that it may in vivo act as a protective reservoir that delivers bioactive molecules at a rate that promotes epithelial migration. Exgel may represent a new treatment principle to promote wound healing. **Key words:** wound healing; topical therapy; chemotaxis; cytokines.

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Hydrogels consist of hydrophilic polymers which are commonly hydrated almost to saturation. They are in general highly biocompatible and are used in many biomedical applications, e.g. in various prostheses, contact lenses and in wound dressings (1).

Hydrogel wound dressings are provided as thin sheet-like plates (e.g. Vigilon®) or in an amorphous form in practical dispensers (e.g. IntraSite® Gel, Smith & Nephew) (2, 3). There are more than ten different brands of hydrogel dressings commercially available today and they have been found useful in wound treatment for debridement of necrotic dry tissue, present for example in chronic and burn wounds, but also for promotion of granulations and re-epithelialisation of clean wounds (4). Hydrogels maintain a moist wound environment, they are transparent, they often provide local analgesia and are gentle to wound and periwound areas during dressing changes. Furthermore, active topical agents such as antiseptics,

antibiotics and growth factors can be incorporated in hydrogels and delivered to wounds at an appropriate rate (5–7). Disadvantages with hydrogels are their limited absorption rate of wound fluid and cohesive strength in contact with body fluids, diminishing their use to less exuding wounds.

In the present study, an experimental amorphous hydrogel with improved physical properties (“Exgel”) was compared with the most commonly used one (IntraSite®Gel) and another hydrogel composed of poloxamer 407 containing 3% hydrogen peroxide in a standardised wound model in the domestic pig under moist wound conditions (8). In addition, complementary in vitro studies were carried out to elucidate possible mechanism(s) of stimulatory action of the new hydrogel dressing.

MATERIAL AND METHODS

Composition and physical properties of the hydrogels

Exgel comprises sodium carboxymethylcellulose (NaCMC), calcium alginate, and water. IntraSite® Gel contains NaCMC, propylene glycol (a humectant and preservative) and water. Poloxamer 407 is a copolymer of polyoxyethylene and polyoxypropylene with stabilised hydrogen peroxide (3%) incorporated and water (Norpharma, Denmark). As indicated in Table I Exgel was the most viscous and closest to isotonic of the three hydrogels, whereas the other two hydrogels were highly hypertonic in comparison to physiological conditions, i.e. phosphate-buffered saline (PBS).

Animal study

Animals. Six pigs, weighing about 60 kg, of a crossbreed of Danish country and Yorkshire, were used. The animals were kept in individual cages at 20°C with 10–12 h of light, fed standard food twice daily and given water ad libitum.

Wounding. The animals were anaesthetised with Sedaperone® (i.m.) and Hypnodil® (i.p.). The back of the pig was shaved and then skin-stripped twice with Comfeel transparent dressing (Coloplast A/S). The skin was washed with soap and 70% ethanol. Four square partial-thickness wounds (2.5 cm × 2.5 cm × 0.04 cm) were made bilaterally on the mid back, using an electrokeratome (Aesculap® GA 630). The wounds were separated by 3 cm uninjured skin, each wound being placed 4 cm ventrolaterally to the dorsal midline. Coagulated blood was removed with sterile gauze. The skin surrounding each wound was wiped with trichlorethane to defat the skin. A thin layer of medical adhesive was brushed onto the surrounding normal skin to improve the adhesion of the occlusive covering.

Dressing of wounds. To eliminate a possible effect of anatomic location on the results, treatments were allocated within two regions on each pig by randomisation. One region comprised the four most cephalad wounds and the other region the four most caudal wounds. One of each of the three different hydrogels was administered to one of the wounds (2 ml/wound) in each region from 5-ml syringes labelled only with the designated wound, covered individually with an adhesive polyurethane film dressing (Tegaderm®, 3M), and the fourth wound was covered with Tegaderm® alone (control). The polyurethane film dressings were covered with gauze, fixed with Fixomull® stretch (Beiersdorf) and a waistcoat was finally put on.

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Table I. Composition, viscosity and osmolality of the hydrogels used in the animal study

Hydrogel	Composition	Viscosity (kpoise) ^a	Osmolality (Osmol/kg) ^b
Exgel	NaCMC, calcium alginate and water	16	0.11
IntraSite® Gel	NaCMC, propylene glycol and water	4	3.18
Poloxamer gel	Co-polymer of polyoxyethylene and polyoxypropylene, stabilised hydrogen peroxide, and water	6.4	2.35

^a Viscosity was measured at ambient temperature using a Brookfield Helipath Spindle T-F at 2.5 r.p.m.

^b Osmolality was measured using a cryoscopic osmometer (Osmat 030, Gonotec). PBS: 0.29 Osmol/kg.

Procedures at 24 and 66 h post-operatively

At 24 h post-operatively, dressed wounds were photographed, dressings removed, wounds cleansed using saline-soaked gauze and new hydrogels administered. New polyurethane dressings were then applied.

At 66 h post-operatively the animals were given 10 ml of Mebumal® intravenously and killed by exsanguination. The time point 66 h was chosen because our experience and that of other investigators is that these partial-thickness wounds treated with occlusive dressings are covered to about 50% by epithelium at this stage (9–11). Epithelial cells migrate from wound edges, hair follicles and apocrine gland ducts at an approximately constant rate to 100% epithelial coverage after an initial lag phase of about 18 h (9–11). The migration rate depends on general and local factors, and thus 50% epithelial coverage is optimal in order to detect significant differences between topical treatments (8–11). Each wound, also including surrounding uninjured skin, was excised and fixed in buffered 4% formaldehyde. Each fixed tissue specimen was cut into four tissue strips of equal width (4 mm) perpendicularly to the dorsal midline prior to routine processing into paraffin. From each paraffin-embedded strip one 5-µm section was stained with haematoxylin-eosin. Epithelialisation was measured on approximately 10 cm of wound length in each wound, using a light microscope (Nikon Optiphot-2) at a total magnification of 100 times. A 10-mm eyepiece micrometer with 100 divisions (Graticules Ltd.) was used to measure the percentage coverage of the total wound length with at least one epithelial cell layer in each section. The mean of the four sections from each wound was used for statistical analysis. All slides were evaluated for epithelium coverage in a blinded fashion.

In vitro studies

Keratinocyte cultures. Keratinocytes were obtained from abdominal skin of a healthy 53-year-old male and expanded according to the protocol of Rheinwald & Green (12) in a DMEM/Ham F-12 medium containing 10% foetal calf serum and grown on a lethally irradiated 3T3 feeder layer. Cells were subcultivated using 0.05% trypsin/0.01% EDTA, switched to serum-free conditions and used between passages 3 and 4 in the in vitro assays.

Effect of amorphous hydrogels and propylene glycol on keratinocyte proliferation. Human keratinocytes (2.5×10^3 cells/well) were added to 96-well plates (Nunc) in 200 µl serum-free medium (KMK-1, Sigma Chemical Co.) which consists of the basal medium MCDB 153 supplemented with bovine insulin (5 µg/ml, final concentration), bovine pituitary extract, EGF (0.1 ng/ml), hydrocortisone (0.5 µg/ml), gentamicin (50 µg/ml) and amphotericin B (50 ng/ml). Calcium concentration of KMK-1 was 0.17 mmol/l, as determined with atomic absorption spectrophotometry. Keratinocytes were grown to about 90% confluence; 110 µl medium was replaced by 20 µl of respective hydrogel diluted in 80 µl medium and by 10 µl of the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU, 10 µM, final concentration) in KMK-1 medium. Control treatment consisted of 20 µl ultrapure, sterile water only (Maxima®, Elga) in 80 µl medium. Proliferation was measured as the incorporation of BrdU during the 24-h exposure period. Incorporated BrdU was immunodetected using

an ELISA-kit (Boehringer Mannheim, Cat. No. 1 647 229) and ΔO.D. (O.D._{370 nm} – O.D._{492 nm}) read by an ELISA reader (Labsystems, Finland). The effect of hydrogels on keratinocyte proliferation was expressed in percentage of control-treated cells.

Chemotactic activity on keratinocytes of wound fluid without and with Exgel components. Chemotaxis of human keratinocytes was induced by 210 µl porcine wound fluid (50%, v/v) in the lower well of a modified Boyden chamber (13). Wound fluid was collected on post-operative day 1, diluted with one part KMK-1 medium containing 0.2 mg bovine serum albumin (BSA)/ml and sterile-filtered (0.45 µm). The Exgel components (NaCMC and calcium alginate mixed in the proportions as in the Exgel) were hydrated in KMK-1 medium containing 0.2 mg BSA/ml to 5% (w/v), using a magnetic stirrer at 400 r.p.m. with Teflon®-coated magnetic spinbars, for 24 h at room temperature. Wound fluid (50%, v/v), wound fluid with hydrogel (0.5% w/v) and hydrogel alone (0.5% w/v) were incubated for 2 h at 37°C prior to the initiation of the chemotactic assay. The recombinant human growth factors epidermal growth factor (EGF, 50 ng/ml (final concentration), Sigma Chemical Co.), interleukin-1α (IL-1α, 100 ng/ml, R&D Systems) and keratinocyte growth factor (KGF, 100 ng/ml, PeproTech) were also tested alone for chemotactic activity. Collagen-coated polycarbonate filters with 8-µm pores (Nuclepore®) were placed on top of the chemoattractants. Trypsinised keratinocytes (4×10^5) were pelleted by centrifugation, suspended in 810 µl KMK-1 containing 2 mg BSA/ml and then added to the upper well. The chambers were incubated for 4 h at 37°C in 5% CO₂ and 95% air. The number of cells that had migrated into the filters were fixed and stained, and the stain was extracted with 0.1N HCl and O.D._{620 nm} measured (13).

Statistical analysis

The in vivo epithelialisation data and in vitro data were analysed by analysis of variance. If this analysis showed statistical significance of treatments, Bonferroni's multiple range test was applied to make comparisons among treatments.

The statistical interpretations were based on statistical tests at 5% -significance level. Data are presented as mean ± SEM (standard error of the mean). Statgraphics® Plus version 6 software was used for the analyses.

RESULTS

Animal study

A striking macroscopic observation was that the Exgel retained its three-dimensional structure on the wounds, whereas the other two hydrogels were completely dissolved at 24 h and 66 h post-operatively. One clinical consequence of this property of the Exgel was that wound fluid did not spread laterally onto surrounding uninjured skin, as was the case for the other two hydrogels and the Tegaderm® control. The wounds treated

with poloxamer containing hydrogen peroxide appeared more reddish on the wound surface than the other treatments.

The morphometric analysis revealed a statistically significantly ($p < 0.05$) enhanced epithelialisation in the Exgel group ($77.5 \pm 4.2\%$ (mean $\pm$ SEM), $n = 12$) compared with the other three treatment groups, while there were no significant differences among the other treatments (Tegaderm[®] control: $62.0 \pm 5.5\%$, $n = 12$; IntraSite[®] Gel: $55.4 \pm 3.1\%$, $n = 12$; poloxamer gel: $53.6 \pm 4.6\%$, $n = 12$).

In vitro studies

Keratinocyte proliferation. No stimulation of keratinocyte proliferation was found with any of the treatments; on the contrary, hydrogels (10%, v/v) inhibited keratinocyte proliferation compared with control-treated cells, although the inhibition was significantly ($p < 0.05$) more pronounced with IntraSite[®] Gel ($70.5 \pm 2.2\%$ of control-treated cells, $n = 10$) and the corresponding concentration of propylene glycol (273 mM, $67.5 \pm 2.8\%$, $n = 10$) than for the Exgel ($85.2 \pm 3.8\%$, $n = 10$). The poloxamer gel containing hydrogen peroxide inhibited keratinocyte proliferation completely.

Chemotaxis. To elucidate further a possible mechanism of action of Exgel a chemotactic experiment using keratinocytes was carried out. Porcine wound fluid induced a significant chemotactic ($p < 0.05$) response in keratinocytes (Fig. 1). EGF (50 ng/ml), IL-1 α (100 ng/ml) and KGF (100 ng/ml) all failed to induce a chemotactic response in the keratinocytes. As seen from Fig. 1, Exgel components added to wound fluid decreased chemotaxis significantly ($p < 0.05$). The observed inhibition of chemotaxis in vitro of keratinocytes may indicate that the chemotactic molecules are sequestered by one or more of the components of the Exgel.

DISCUSSION

Hydrogel dressings are advantageous in many respects in wound management. In this study three amorphous hydrogels

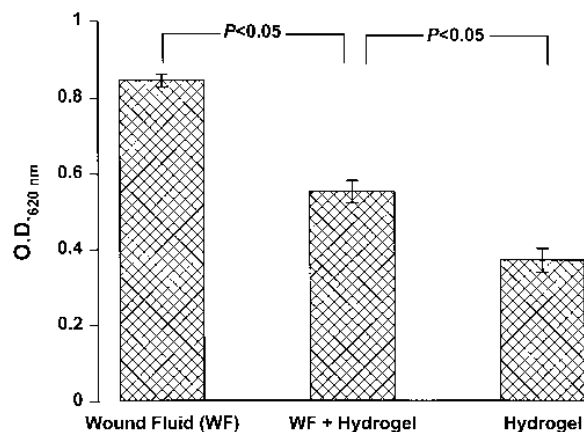


Fig. 1. Chemotactic activity on keratinocytes of wound porcine wound fluid (50%, v/v) alone, wound fluid pre-incubated with hydrogel (0.5%, w/v) components or hydrogel (0.5%, w/v) components alone. The chemotactic activity is expressed as absorbance at 620 nm, which is a direct measure of the number of keratinocytes that have migrated into the filter (13). Each bar represents the mean $\pm$ SEM of three measurements.

were compared in a validated and standardised experimental wound healing model (8–11). A stimulatory action on wound healing was seen with the experimental hydrogel (Exgel), whereas the two other hydrogels displayed no effect on epithelialisation in comparison with occlusive therapy.

The conclusions about the effects of treatments on epithelialisation are based on a single time-point morphometric evaluation by light microscopy of only partially epithelialised wounds. Eaglstein & Mertz (10) found a correlation between the number of completely healed wounds and percentage epithelialisation using the same experimental model as ours. Therefore, treatment with Exgel would also most likely result in an earlier complete wound healing of these types of wounds.

Furthermore, a major clinical difference observed among the hydrogels was the resilience to disintegration of the Exgel, which retained its three-dimensional structure over an extended treatment period. This resulted in less lateral spreading of wound fluid. This finding may indicate a decreased risk of skin maceration.

It is noteworthy that Exgel accelerated epithelialisation in comparison with occlusion alone. In vitro, such stimulatory direct action on keratinocyte proliferation was not found with Exgel. However, the porcine wound healing model used primarily measures the effects of treatments on migration rather than on proliferation of keratinocytes (9). To mimic keratinocyte migration an in vitro chemotaxis experiment using the Boyden chamber was performed. As reported earlier (14), wound fluid possessed chemotactic activity on keratinocytes, although the chemotactic molecule/molecules in the wound fluid remain unknown. For example, in another study IL-1 α was chemotactic only if the keratinocytes were freshly trypsinised but not if they were cultured in advance (15). When the polymeric components of the Exgel were added to wound fluid, in vitro conditions that simulate hydrogels applied to a wound, the chemotactic response to the wound fluid was decreased (Fig. 1). Thus, one possible mechanism of action is that in a wound, Exgel may function as a protective reservoir for endogenous biologically active molecules, which are released at a rate optimal for epithelial migration due to its high cohesive strength. In fact, glucosaminoglycans, which are negatively charged polysaccharides as NaCMC, bind growth factors (16).

In another study, the importance of the carrier system on wound healing was examined using transforming growth factor- β 1 (TGF- β 1) incorporated in different hydrogels (7). In that study the effect of TGF- β 1 on wound healing varied depending on the type of hydrogel used. Furthermore, we have earlier shown that a hydrogel sheet dressing increased collagenase levels in wounds, which might facilitate epithelial migration (2).

The IntraSite[®] Gel and the poloxamer gel contain bacteriostatic agents, propylene glycol and hydrogen peroxide, respectively. Exgel does not contain the humectant propylene glycol in contrast to IntraSite[®] Gel. The more pronounced inhibition of keratinocyte proliferation with IntraSite[®] Gel was possibly due to the presence of propylene glycol (17). The use of hydrogen peroxide in wound management has been debated, without any consensus having been reached. Our results using 3% stabilised hydrogen peroxide indicate no inhibitory effects on wound healing. Actually hydrogen peroxide treatment appeared to increase blood perfusion, as reported previously for ischaemic guinea pig wounds (18).

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