Effect of a DACC dressing on the growth properties and proliferation rate of cultured fibroblasts

- **Objective:** To study the morphology and proliferation of cultured fibroblasts in combination with an experimental wound-healing model using cultured fibroblasts, with and without the presence of a hydrophobic, dialkyl carbamoyl chloride (DACC) dressing (Sorbact; Abigo Medical AB).

- **Method:** Human dermal fibroblasts were cultured and cell morphology and viability were investigated. Proliferation was investigated using an XTT assay. An experimental wound-healing model was employed, whereby mechanical damage was inflicted to the surface of cultured fibroblasts. The healing and closure of the wound was then monitored with and without the presence of the DACC dressing.

- **Results:** Fibroblasts did not adhere easily to the dressing material. The presence of the DACC dressing increased the average proliferation rate of cultured fibroblasts by 50% compared with the untreated control medium ($p < 0.05$). The DACC dressing significantly increased the healing rate by more than 100% after 72 hours ($p < 0.05$) in the experimental model of wound healing, compared with the medium only.

- **Conclusion:** The enhanced wound healing observed in different types of wounds using the DACC dressing might be explained by an increase in cell growth and proliferation rate of cells in the wound area.

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cell culture; experimental model; fibroblasts; dialkyl carbamoyl chloride; hydrophobic; wound healing

hydrophobic dressings present an alternative for reducing the microbial load in open wounds without enhancing nosocomial spread, with a documented clinical effect for a variety of wound types. The mechanism of action is based on a hydrophobic interaction between the hydrophobic coating of the dressing, such as dialkyl carbamoyl chloride (DACC), and microbes whose surfaces contain water-repellent molecules. Existing data are limited, but indicate that the pathogenic microorganisms bind to the hydrophobic dressing and are thus eliminated from the wound, without any known side effects.

However, for several years, both subjective and objective reports have suggested that treatment with hydrophobic antimicrobial dressings promotes healing. Recent case reports indicated healed or improved dermal fungal infections, both inter-digital (athlete’s foot) and in skin folds. In most of the cases, the wound was observed to have healed or improved within 8 days; however, the results indicate that the binding and inactivation of pathogenic microorganisms may not be the only mechanism resulting in enhanced wound healing.

To date, no studies have investigated other possible mechanisms, apart from hydrophobic properties and binding of pathogens, that could be involved in enhanced wound healing and/or in activating the wound-healing process in wounds where healing is stalled or impaired.

This in vivo experimental pilot study was, therefore, performed to investigate the effects of a DACC-coated dressing (Sorbact; Abigo Medical AB) on cultured fibroblasts. Primary endpoints were effects on morphology and proliferation of the fibroblasts. An experimental wound-healing model was also used to investigate wound closure and calculate wound-healing rate.

**Method**

**Materials**

Commercially available human dermal fibroblasts (CCL-110; ATCC/LLC Standards) were cultured at 37°C in an atmosphere of 5% CO₂, in humidified air, in a CO₂ incubator (Forma Science; AB Nilolab), using Eagle’s minimal essential medium with Earle’s salts (EMEM; Sigma-Aldrich) supplemented with 10% foetal calf serum (FCS; Sigma-Aldrich). Cell cultures were handled aseptically, using a laminar airflow bench (folten 2448; Nilolab) and stored in a liquid nitrogen tank.

Fibroblasts were initiated in culture, as previously described by Falk. Briefly, ampoules of frozen cells were thawed by submerging into a 37°C water bath for 30 sec. Cells were re-suspended in 10 ml preheated complete medium, and centrifuged at 2600 g for 10 min. The supernatant was then discarded, and
the cell pellet was further re-suspended, with 5ml complete medium, into 25cm² cell culture flasks (Cell+; Sarstedt). At confluence, cells were further subcultured using trypsin/EDTA (0.5/0.2%) solution (Sigma-Aldrich).

Culture media were changed three times a week. Culture flasks and culture plates (Falcon/BD; Franklin Lakes) were used for experimental setups. Cells were regularly monitored using an inverted microscope (Axioskop 25; Carl Zeiss, Inc.) connected to a digital image processing software (Axiovision; Carl Zeiss, Inc.). This equipment has been used extensively to analyse and monitor cell cultures.¹⁵

Sterile 45cm² Sorbact compresses (Abigo Medical All) were used in the experiments. In order to fit in the different culture vessels, the compresses were cut into smaller pieces using a sterile 5mm-diameter disposable biopsy punch (Miltecs GmbH) for 24-well plates (1.88cm²), and an 8mm punch for 12-well plates (3.83cm²), under sterile conditions.

**Study protocol**

Prior to the start of the experiments, cells were cultured to approximately 50% confluence and then pre-incubated for 24 hours in a medium containing 13% FCS. All wells were gently washed with medium containing 1% FCS, with all experiments run using medium with 1% FCS only. Cell count was done prior to the start of the experiment to verify that there was no difference in cell count between the different wells. Between measurements, cell cultures were incubated at 37°C. Each experiment was run twice.

**Morphology and viability** To investigate the effect on the morphology of cultured fibroblasts, cells were grown into subconfluence and then sub-cultured into 12- or 24-well plates (Sarstedt). In these experiments, the compresses were used both cut and placed into the medium (24-well plates), and submerged in the medium (12-well plates). The hydrophobic properties of DACC-coating resulted in difficulties submerging the compresses; however, this was solved by mounting the compresses between two sterile acrylic rings before submerging them into the culture dish with medium.

The viability of the cells was then studied during subculture, using fibroblasts dispensed onto the dressing, to investigate the attachment of cells to the material and their further viability. A total of 12 wells were used for each setup, with six wells used for the dressing material and six wells with medium only, as a control.

**Proliferation** Fibroblast proliferation was studied using a sodium salt that is metabolically reduced in living cells to produce a stable orange colour (XTT assay, Sigma-Aldrich), which can be analysed in a spectrophotometer. The colour change is proportional to the mitochondrial dehydrogenase activity, resulting in an effective technique for measuring cell toxicity,¹⁶ or cell proliferation.¹⁷ The colour change was measured using a 96-well plate reader (VMax Kinetic ELISA; Molecular Devices) and software was used to calculate the absorbance at 450nm (Softmax Pro, Molecular Devices).

During all proliferation studies, a medium without phenol red was used to reduce the red background signal in the XTT assay. Absorbance at 450nm and 650nm was measured; levels from the medium were subtracted from those in the control wells, which contained only culture medium. A total of eight wells were used in each setup for proliferation: four wells containing fibroblasts in the presence of compresses of area 5mm² placed in the medium, with four plates serving as untreated controls.

**Wound-healing model** Cultured fibroblasts were grown until confluence at the start of the experiment. A mechanical injury was created by scraping the monolayer of cultured cells with a 10ml sterile plastic pipette (DIOST, Gilson, Inc.), forming a cross, a technique that has been used previously with both mesothelial cells, mimicking damage to the peritoneal surface,¹⁸ and in cultured foreskin fibroblasts.¹⁹

The resulting damage was measured with a calibrated size marker at several time points, using the image processing software. In a clinical situation, wound repair is generally measured through the reduction in area; however, in this in vitro model, with a monolayer of cells, we found it more correct to identify cell proliferation and migration by measuring the distance between the cells. To accomplish this, a line was drawn parallel to the wound edges, with multiple lines then drawn perpendicular to this, across the damaged area. This allowed a mean value for the length of the damaged area to be calculated. To reproduce measurements between incubation times, calculations were always made in the same area. Creating a cross in each culture well made it easy to identify this area by locating the centre of the cross and always beginning in the upper right arm of the cross.

Healing rate was calculated by dividing the restored distance by the time between each measurement. Measurements were performed at 1, 3, 5, 6, 24, 32, 48, 56 and 72 hours, after creating the mechanical damage. In the wound-healing model, the compresses were cut into small pieces using the 5mm biopsy punch. A total of 12 wells was used, with six wells used for the dressing material and six wells with medium only. Care was taken not to contaminate the culture wells with dressing debris or fibres.

**Statistical analysis** The non-parametric Kruskal-Wallis test was used to detect overall differences, with the Mann-Whitney U test performed between individual...
groups. A p-value < 0.05 was considered statistically significant. All calculations were performed using StatView software (v5.0, Abacus Concepts).

Results

Established fibroblastic cultures were seen in all experiments. Cells could be cultured, subcultured and re-frozen in liquid nitrogen without affecting growth performance.

Effect on morphology

The presence of the dressing material promoted the growth properties of the cultured fibroblasts compared with cells in the control medium (Fig 1). However, cells did not easily adhere to the surface of the material (Fig 1a), even though the growth of cells was more efficient in wells containing the dressing (Fig 1c,e), compared with cells cultured with medium only (Fig 1d,f). Single fibroblasts did not easily attach to the dressing surface, with none of the cells that did adhere surviving to multiply into an established colony (Fig 1a). It is notable that damaged parts of the material, or single fibres separated from the compress, were easily covered with fibroblasts (Fig 1b), an observation that was also reproduced in a separate pilot study.

Effect on proliferation

The presence of the dressing material improved the proliferation of fibroblasts compared with cells cultured with the medium only. In repeated experiments (n=8), cells cultured for 24 hours in the presence of the dressing had an elevated proliferation rate compared with the control group (195 ± 13% vs 100 ± 62%; p=0.0028; Fig 2).

Wound-healing model

Mechanical damage to the fibroblastic surface resulted in a denuded injury averaging a distance of 530 ± 40 μm between the edges, with no significant difference between dressing and control wells (p=0.15). In all experiments using the dressing (n=12), the mechanical injury was fully recovered within 72 hours, while the cell culture wells with the control medium (n=12) failed to reach complete healing in the same period. The presence of the dressing caused the fibroblasts to migrate across the damaged area more rapidly than cells in medium only. This was illustrated with photomicrographs, with a greater number of fibroblasts repairing the denuded area in cells with the dressing, compared with cells treated with only the medium (Fig 3).

The healing rate was elevated in fibroblasts in the presence of the dressing compared with cells in the medium alone. This difference was most pronounced in the healing rate after 72 hours (n=6, p=0.043), as illustrated in Fig 4. For total fibroblastic restoration, a significant difference between the dressing and the untreated control medium was observed between 56 and 72 hours (Fig 5).

Discussion

This pilot study suggests that, along with the basic mechanism of the hydrophobic action of binding and inactivating microorganisms, there are other possible mechanisms affecting the proliferation rates in cultured fibroblasts. There is limited clinical evidence of enhanced wound healing using hydrophobic antimicrobial dressings in the literature, with most data limited to case reports. This in vivo study demonstrated that cultured human dermal

![Fig 1. Phase contrast microscopy of the DACC dressing and cultured fibroblasts. Fibroblasts did not adhere easily to the material, (a), unless it was damaged, (b). The growth properties were improved in the DACC dressing group, (c,e) compared with controls, (d,f).](image-url)
fibroblasts were affected in the presence of the Sorbact material. The subjective initial indications of improved culture conditions in the presence of the material were later demonstrated objectively with an increase in proliferation rate, using the XTT assay. Cultured fibroblasts showed an increased proliferation rate in the presence of the dressing compared with control cells, cultured with the medium alone.

Musin et al. described the improvement in patients with severe pressure ulcers, using DACC-coated dressings. They demonstrated an improvement in the colour of the ulcer bed, as well as an increase in cell debris dissolution, concluding that use of the hydrophobic antimicrobial dressing could shorten treatment time by binding microorganisms to the material, and also reduce the use of antibiotics for these patients. By binding microorganisms to the DACC-coating, the healing process was improved and infections were eliminated or reduced.

The increase in cell proliferation observed in this study suggests additional properties of the dressing in the presence of living cells. Therefore, its key effects may not only be antimicrobial, but also to stimulate proliferation of cells involved in wound closure. However, to identify whether this effect is due to the Sorbact material itself, or the DACC coating component, will require further investigation to demonstrate if the material releases any active components affecting the environment.

The wound-healing model, with mechanical damage, could suggest the dressing significantly increases wound healing compared with control cells. In an experiment performed on mesothelial cells, Yung and Davies used a glass rod to mechanically scratch the cells, to create a damaged surface, while Longaker et al. also used the 'cray' model on cultured fibroblasts. However, an earlier pilot study, on both fibroblasts and other cells (unpublished data), showed that the plastic surface of the culture plates was easily damaged using the glass rod; therefore, a plastic pipette was used instead, allowing mechanical damage to be created in a reproducible way, without damaging the culture surface.

In their study, Longaker et al. monitored the cells until the damage was fully restored, finding the edges had completely closed after approximately 16 hours. This differs from the results observed in this study, where the damaged surface was restored between 48 and 72 hours. The reason for this is not known; however, it is possible that the damage in our model was greater than in the model previously described. Moreover, the use of 10% FCS in the same model, compared with 1% FCS in the present study, is another possible factor affecting the growth properties, and restoration time, of the damaged area.

In the present study, fibroblast cells did not easily attach to the DACC-coated surface. Only single cells were found to attach, and none of these cells survived to multiply into an established colony of cells; this could be due to the hydrophobic properties in
Fig 5. Restoration of damaged fibroblast surface

<table>
<thead>
<tr>
<th>Time after mechanical damage (hours)</th>
<th>Width of restored surface (µm)</th>
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<tbody>
<tr>
<td>&lt;1</td>
<td>-100</td>
</tr>
<tr>
<td>1-3</td>
<td>0</td>
</tr>
<tr>
<td>3-6</td>
<td>100</td>
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<td>6-24</td>
<td>200</td>
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<td>24-32</td>
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<td>400</td>
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<td>48-56</td>
<td>500</td>
</tr>
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<td>56-72</td>
<td>600</td>
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As at 48 and 56 hours after mechanical damage, there was a tendency for a difference in the restored surface. By 72 hours, the restoration differed significantly between the DACC group and untreated medium control (p<0.001, n=6).

The absorbent dressing in contrast, damaged parts of the dressing, or single fibres, in the culture plates were quickly covered with existing fibroblasts. This suggests that the dressing should be used intact, as it could otherwise cause cells in the centre of the closing wound to adhere to the damaged dressing, leading to trauma at dressing change.

Limitations
The experimental model described has several limitations. The use of a cell line with fibroblasts does not fully represent the components in the healing wound. Surrounding extracellular factors, and other cells and growth factors in the nearby tissue, which normally affect the wound in vivo, were not present. Further studies will elucidate the role of other cells, such as keratinocytes, in the healing model. However, despite being a simplified model of a wound closure, this is an important initial finding and the use of Sorbact material in a similar model has, to our knowledge, never before been demonstrated.

Conclusion
To our knowledge, this is the first experimental study showing an increase in cell proliferation and migration in an experimental cell culture model, using the Sorbact dressing. This could suggest additional properties of the dressing in the presence of living cells, along with the hydrophobic binding of pathogens, which could prove important in a clinical context.

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