MUCOPOLYSACCHARIDES FROM PSYLLIUM INVOLVED IN WOUND HEALING*

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Summary: Mucopolysaccharides derived from the husk of psyllium (Plantago ovata) have properties beneficial for wound cleansing and wound healing. Recent studies indicate that these mucopolysaccharides also limit scar formation. Our in vitro and in vivo studies aimed to investigate the mechanisms involved, e.g., fluid absorption, bacterial adherence and in vitro stimulatory effects on macrophages, which are pivotal in wound healing. The mucopolysaccharides contained in a sachet (Askin®Cavity) or in a hydrocolloid mixture (Askin®Hydro) were found to have a gradual and sustained absorbency over a period of 7 days, amounting to 4-6 times their weight in water. The swelling index was 9 mm after 312 h. Adherence of wound bacteria to the mucopolysaccharides started after 2 h and was more pronounced after 3 h. Semiquantitative measurements of bacterial adherence used centrifugation and subsequent optical density determinations of supernatant. These confirmed the strong adherence potential of psyllium particles. Lactic acid dehydrogenase staining of pretreated cultured human skin explants did not reveal toxicity of the mucopolysaccharides derived from psyllium husk. Langerhans’ cell migration from the epidermis was negligible and interleukin-1β expression in the explants was not significant, supporting the very low allergenic potential of psyllium. The characteristics of mucopolysaccharide granulate derived from psyllium husk in Askin®Cavity and Askin®Hydro related to fluid absorption, bacterial adherence, biocompatibility, stimulation of macrophages, irritancy response and allergenicity showed an optimal profile, supporting the good clinical performance of wound healing products containing psyllium husk.

Introduction

Psyllium husk or isphagula husk is the coat (epidermis) of the seed of Plantago ovata, Plantago psyllium, or Plantago indica. The husk is composed of mucopolysaccharide complexes, including polysaccharides chains with side chains of fucose, glucose,
galactose, rhamnose and arabinose (1, 2). It is presented in powder form, which swells rapidly in water, forming a stiff mucilage (3). For decades, psyllium has been widely used as a laxative to treat constipation or as a bulky material to treat diarrhea. It has even been used to treat peptic ulcers.

We have demonstrated the efficacy of this substance in chronic leg ulcers. Psyllium-containing pads led to better cleaning of yellow necrosis and quicker red granulation tissue formation than saline gauzes (4). The same pads also proved to decontaminate infected chronic wounds, such as leg ulcers (5). Recently, we found that a psyllium-containing hydrocolloid dressing produced a better effect on wound healing, e.g., less atrophy, than a polyurethane adhesive film dressing (6). The psyllium-containing hydrocolloid dressing performed better in adhering to the skin, even under damp conditions, so that no leakage took place. This was confirmed in another study, which demonstrated better control of the malodor (7).

At the same time, the dressing could be removed from the skin without damage or pain (6). The hydrocolloid/psyllium dressing was able to adhere to the skin for about 12 days without irritating it.

The beneficial effects of psyllium-based wound dressings are determined by a multitude of factors. The aim of the present study was to determine the characteristics of mucopolysaccharide granulate derived from psyllium husk in Askina®Hydro and Askina®Cavity related to fluid absorption, adhesion to skin, bacterial adherence, biocompatibility, stimulation of macrophages, toxicity and allergenicity.

The profile of the psyllium husk-derived mucopolysaccharides of Askina®Hydro and Askina®Cavity was very favorable.

Materials and methods

Fluid absorption. The fluid absorption of psyllium husk granulate containing hydrocolloid dressing (Askina®Hydro; B. Braun Hospicare, Sligo, Ireland) was measured by the increase in volume of a sample of gum per unit of area, which was in contact with a saline solution for 4 h. A glass cylinder (40 mm height; 29 mm diameter) was placed on top of a hydrocolloid disk (40 mm diameter; B. Braun Hospicare, Sligo, Ireland). The disk was exposed to a saline solution over a specified time period. The subsequent increase in weight was calculated per meter:

\[
(\text{final weight} - \text{initial weight}) \times 1,522
\]

where 1,522 was the number of cylinders in 1 m² (unit of measurement: g/m). In addition, the swelling index was estimated after 1 h and consecutively up to 312 h. The swelling index represents the increase in dressing thickness resulting from the fluid uptake and varies with time. A glass cylinder (40 mm height; 29 mm diameter) was placed on top of a hydrocolloid disk (40 mm diameter). The disk was exposed to a saline solution over a specified time period and the subsequent increase in thickness of the sample was calculated (unit of measurement; mm). A second test (fluid handling) was carried out according to the British Pharmacopoeia (Fluid Handling: Monograph Semi-permeable Hydrocolloid Dressings; p. 1943; 1993 Edition of British Pharmacopoeia, Publisher HMSO) for semi-permeable hydrocolloid dressings.

The absorbency of the tested product was determined by simply immersing the granulate of the psyllium husk contained in a porous polypropylene sachet (Askina®Cavity; B. Braun Hospicare) in a saline solution and weighing it at various intervals for up to 7 days. A second test was performed in which the sachet was allowed to absorb saline under conditions of compression. The dressing was weighed and placed in a paddington cup (16 ml). The cup was placed into a deep tray and a perforated perspex sheet was placed on top to ensure that the expansion of the dressing could not exceed the volume of the cup. A weight was placed on top of the perspex sheet to ensure that the apparatus...
remained immersed in a saline solution. The saline solution was poured into the tray to cover the perspex sheet. After a specified time period the dressing was removed and allowed to drain freely for 5 min; the dressing was then reweighed (unit of measurement: g).

**Microscope examination.** To visualize the swelling of psyllium husk particles, a microscope was used; particles were observed under dry and wet conditions.

**Adhesion.** This method determined the tack of a sample of Askina®-Hydro to a stainless steel ball. A second test was carried out according to the British Pharmacopoeia (Adhesiveness: Appendix XXI) for semipermeable hydrocolloid dressings. A sample was firmly attached to a stainless steel plate with the adhesive side of the dressing exposed. A stainless steel ball (40 mm diameter) was brought to rest on the dressing for 15 sec. The adhesiveness of the dressing was calculated as the force needed to remove the steel ball (unit of measurement: gf).

Askina®-Cavity measures the low adherence of the pad to the wound due to the gel formation around the sachet. This test method simulates the adherence between a dressing and the surface of a drying wound. Silicone rubber was placed on the side of the dressing in contact with the wound and was allowed to dry. The force required to remove the silicone rubber from the dressing was measured (unit of measurement: gf).

In vitro bacterial adherence to psyllium husk granulate. Bacteria are generally unable to hydrolyze the polyxyloose backbone of the psyllium mucopolysaccharides (and hence to use it as a growth substrate). The only known exception is Bacteroides ovatus (8).

The adherence of bacteria to polysaccharides was evidenced in both native methylene blue stained preparations under phase contrast and normal light microscope. The semiquantitative measurement of adherence was proceeded by determining the optical density of bacteria/mucopolysaccharide mixtures after centrifugation.

The bacterial strains used in this study were obtained from the initial cultures taken from patients with leg ulcers admitted to the Academic Medical Center, Amsterdam, The Netherlands. These cultures showed a high degree of growth of Staphylococcus aureus, enterococci and commensal flora. Salmonella typhimurium was used as a reference strain because earlier research showed that these pathogens do not adhere to psyllium. Four solutions were made of psyllium in Nutrient Broth (Oxoid CM1/2: TNO/CIVO, Zest, The Netherlands) and tap water (1 g per 25 ml). Solutions 1 and 2 were mixed with bacterial suspensions containing wound bacteria. Solutions 3 and 4 were mixed with the S. typhimurium suspension.

In general, bacterial suspensions contained approximately 5 million colony forming units per ml.

Qualitative adherence. Equal amounts of the solutions and bacterial suspensions were homogenized and incubated aerobically at 37°C (+0.1°C) in a water bath. The suspensions were gently shaken for 15 min. Samples were taken at regular intervals from the bacteria-psyllium mixtures just before and during incubation. Adherence to psyllium particles was determined in both native preparations under phase contrast microscope and methylene blue stained preparations under normal light microscope with 400 and 1,000 times magnification (Zeiss, 40 and 100 phase-contrast and standard oil immersion lenses).

Semiquantitative adherence. Centrifugation was used to separate the bacteria-psyllium mixtures into a precipitation fraction that contained bacteria adhering to psyllium and a supernatant fraction with only free bacteria. The psyllium-bacteria mixtures were centrifuged (Labofuge GL, radius = 150 mm;
Heraeus, Paris, France) in 10 ml tubes at 600 rpm (60 g) for 15 min. Before and after centrifugation the optical density of the mixtures and the supernatants were measured (wavelength: 541 nm). The difference in optical density before and after centrifugation was a semiquantitative measure for the number of bacteria trapped by psyllium particles. The precipitates were resuspended in the original volumes to measure the optical density. In addition, native and methylene blue stained preparations were made for phase-contrast microscopy to verify the findings.

**Biocompatibility and macrophage stimulation.** The tolerance (biocompatibility) of the psyllium granulate was tested in female adult Yorkshire pigs (Vending, Amsterdam, The Netherlands) by subcutaneously injecting a sterile suspension intradermally and evaluating the infiltrate at 1, 7, 21 and 42 days in biopsies. Four adult Yorkshire pigs were used in this experiment. The animals were kept in metal pens. The rooms were kept at constant temperature and humidity (+10%). The animals were given standard feed and had access to water ad libitum. The animals were injected subcutaneously with 2 ml (1 mg/10 ml) of sterile psyllium husk suspension (γ-irradiated) in four sites to the left and right of the spine. After 24 h, 7, 21 and 42 days, full thickness biopsies (8 mm) were taken, using 1 ml of procainehydrochloride (20 mg/ml) for local anesthesia.

For histological examination, hematoxylin/eosin staining and a CD68 monoclonal antibody (Dako KP1, Glostrup, Denmark) as a macrophage marker were used on frozen sections. The migration of macrophages was monitored both in the animal model (Yorkshire pig) and in a skin explant method (see below).

Toxicity. Acute skin irritation for intact and abraded skin was tested in male New Zealand White rabbits (ANKI, Someren, the Netherlands) weighing 2.5±0.2 Kg.

The acute skin irritation test described in ISO 10993-10:1995 (E) was performed with the hydrocolloid-containing material to determine the skin irritation response of rabbits. The polar and nonpolar test and control extracts were applied to the skin of three rabbits and remained in contact with the skin for a period of 24 h.

A tissue culture cytotoxicity test, using L929 cells (European Collection of Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, UK) - the indirect (agar diffusion) method - was performed to test for the presence of leachable, toxic substances from solid materials. Triplicate samples of the material to be tested were placed on top of an agar/medium overlay above a monolayer of mouse fibroblasts (L929 cells). This test follows ISO 10993-5 standards. In addition, the irritancy of psyllium husk extracts was monitored in skin explants (see below).

In these experiments we investigated the irritancy of the product (2%, 5%, 10% viscous solution), the gel of the product and also preswollen blocks (6 mm) by putting it on the cultured human explants. After 24 h, the explants were analyzed immunohistochemically for toxicity by lactic acid dehydrogenase (LDH) staining.

**Allergenicity.** The guinea pig sensitization (maximization) test on Askina-Hydro according to ISO 10993-10:1995 (E) was performed to see whether sensitization (delayed contact hypersensitivity) occurred following injection and topical application of psyllium extracts. The study consisted of an induction phase and a challenge phase, involving topical application of the test material extracts to a previously unexposed site on the animal. Thirty female Dunkan Hartley guinea pigs (Breeding Unit of Biological Laboratories Ltd., Mayo, Ireland), weighing 411.65 ± 31.95 g at day 0, were used.

The allergenicity of psyllium husk granulate and mucilage was also measured in an ex vivo human skin organ culture.
Epidermal Langerhans’ cells and cytokines play a critical role in the initiation phase of contact hypersensitivity reactions in the skin. Most of the studies of these aspects have been performed in animal models. Short-term human skin organ cultures, in which Langerhans’ cells preserved their characteristics and distribution within the epidermis, were used to examine the time course effects of possible contact allergens (psyllium granulate and psyllium mucilage) on human Langerhans’ cells in situ and to determine whether these effects were mediated by cytokines. We have previously described the methods in detail (9, 10).

Skin specimens. Fresh human adult skin from normal healthy individuals was obtained from patients undergoing breast or abdomen reduction surgery in the Department of Plastic Surgery, Academic Medical Center, Amsterdam, The Netherlands. Specimens were thoroughly washed with RPMI-1640 supplemented with 1% penicillin-streptomycin (GIBCO Life Technologies Ltd., Paisley, Scotland) and processed immediately for culture.

Skin organ cultures and experimental procedures. A Trowell-type skin organ culture method was employed for the time course analysis of the hapten treatment of human skin during culture. The procedure and assembly of the skin organ culture were performed according to our previous reports (9, 10).

Cytokines and antibodies. We bought human recombinant interleukin-1β (GenZyme, MA, USA). We used primary monoclonal antibodies against CD1a (Ortho Diagnostic Systems, High Wycombe, UK), HLA-DR (Becton Dickinson, Oxford, UK) and CD68 (Dako K.P., Glostrup, Denmark).

Hapten treatment of human skin in organ culture. Nontoxic concentrations of each chemical compound were evaluated by examining the intact morphology of Langerhans’ cells as well as the epidermal architecture after 24 h of skin application of chemical compounds.

Tissue processing and histological analysis. Cultured skin explants treated with hapten were harvested at various time intervals and snap-frozen in liquid nitrogen. The tissues were stored at -70°C until sectioning. Serial cryostat sections (6 mm thick) were cut from each skin explant, air-dried and fixed in cold acetone for 10 min. Some sections were frozen unfixed until used for the in situ cytokine analysis. Approximately three sections from each explant were stained with hematoxylin and eosin for histologic evaluation and others were processed for immunohistochemistry.

Quantification of Langerhans’ cell migration in situ. We evaluated the migration of epidermal Langerhans’ cells by monitoring the changes in the quantity of CD1a and HLA-DR epidermal dendritic cells as a measure of possible allergenicity of the product that might sensitize the host. Furthermore, we performed a preliminary examination of the effect of the products on the changes in macrophage-type (CD68+) cells. The cells that were localized on or near the basement membrane, up to one layer above the basal keratinocytes (migratory area), were considered migrated Langerhans’ cells.

Data analysis. The mean ± SD was calculated for data from various experiments. Statistical analysis using Student’s t-test was performed and the results were considered significant when p < 0.01.

Results

Fluid absorption. Mucopolysaccharides in a sachet (Askina®Cavity) showed a gradual absorption over a 6-day period (Fig. 1). When the absorption of the sachet with mucopolysaccharide granulate was
allowed to take place under conditions similar to those found in the clinical situation of compression therapy, there was a gradual absorption of six times its own weight (3.8 g) in 6 days (Fig. 2).

The mucopolysaccharides in a hydrocolloid mixture (Askina®Hydro) appeared to have a gradual and sustained absorbency over a period of 7 days in which four to six times their weight in water was found (Fig. 3). The swelling index was 9 mm after 312 h (Fig. 4).

The results were obtained using the British Pharmacopoeia monograph for semipermeable hydrocolloid dressings (Table I).

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**Fig. 1** Askina®Cavity: Absorption without compression.

**Fig. 2** Askina®Cavity: Absorption with compression.
Table 1 British Pharmacopoeia monograph for semi-permeable hydrocolloid dressings

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<th>Characteristics</th>
<th>British Pharmacopoeia requirement</th>
<th>Askina®Hydro result</th>
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</thead>
<tbody>
<tr>
<td>Fluid handling</td>
<td>The average result for 5 samples shall not be less than 1.5 g/10 cm² per 24 h</td>
<td>Pass 4.99 g/10 cm²</td>
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<tr>
<td>24-h test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid handling</td>
<td>The average result for 5 samples shall not be less than 2.0 g/10 cm² per 24 h</td>
<td>Pass 5.81 g/10 cm²</td>
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<td>48-h test</td>
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![Absorption vs. Time Graph](#)

**Fig. 3** Askina®Hydro saline: Absorption vs. time.

![Swelling Index vs. Time Graph](#)

**Fig. 4** Askina®Hydro: Swelling index vs. time.
Microscopic examination. Figure 5a shows a dry psyllium husk particle comprising about 50-50 cells. Figure 5b demonstrates the swelling process of the polysaccharides. When exudate was absorbed by psyllium granules, the polysaccharides swelled while retaining their structure. Cells remained anchored to the husk, allowing stable gel formation.

Adhesion. The dry ball adhesion of a 30-mm diameter disk of Askina®Hydro containing mucopolysaccharides was 1,008 gf. This was 350 gf under wet conditions. The results were obtained using British Pharmacopoeia monograph for semipermeable hydrocolloid dressings (Table II).

For the mucopolysaccharide containing Askina® Cavity sachets, the test showed that after a 7-h period enough gel had formed to suppress adherence of the silastic foam to the dressing.

Bacterial adherence:

Qualitative adherence. Adherence of wound bacteria to psyllium particles started after 2 h of incubation. The adherence was even more pronounced after 3 h. Mixtures 3 and 4 showed no adherence after 3 h of incubation, thus confirming our earlier research data. The methylene blue stained slides confirmed the findings of the native preparations (Fig. 6).

Semi-quantitative adherence. Before and after incubation, the optical densities of the mixtures and the supernatants were measured in duplicate. The results indicated that mixtures 1 and 2 showed strong adherence capability; the supernatants were almost clear of wound bacteria. Mixtures 3 and 4 showed no adherence (Table III).

Biocompatibility and macrophage stimulation. The mucopolysaccharides injected intradermally in adult Yorkshire pigs demonstrated a mild infiltrate after 24 h. After 7 days, this consisted almost entirely of macrophages, which were lying around the psyllium husk particles (Fig. 7). After 21 and 42 days, no par-

<table>
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<th>Characteristic</th>
<th>British Pharmacopoeia requirement</th>
<th>Askina® Hydro result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesiveness</td>
<td>Maximum leakage 2.5 mm after 30 min at 36-38°C</td>
<td>Pass</td>
</tr>
<tr>
<td>Appendix XXH</td>
<td></td>
<td>0 mm slip</td>
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Table III  Optical densities before and after 3 h of incubation of mixtures (M) and supernatants before and after centrifugation (C).

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Before incubation</th>
<th>After incubation</th>
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<tr>
<td></td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>0.98</td>
<td>0.83</td>
</tr>
<tr>
<td>2</td>
<td>1.13</td>
<td>0.97</td>
</tr>
<tr>
<td>3</td>
<td>1.15</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>1.05</td>
<td>0.93</td>
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Fig. 6. Adherence of wound bacteria to psyllium particle.

Fig. 7. Macrophages around psyllium husk particles.

ticles were detected. The infiltrate had resolved completely after 42 days. Giant cell granuloma formation did not occur.

Toxicity. Both the extract from the hydrocolloid sheet and the polypropylene sachet containing psyllium husk granulate yielded a primary irritation index of 0.11. This may be categorized as a negligible response and was considered to pass the acute skin irritation test.

The test material (psyllium/hydrocolloid extract) was not toxic in the tissue culture cytotoxicity test using mouse fibroblasts (L929 cells). The negative control (filter disks, prewet with culture media) was not toxic to L929 cells under the conditions of this test. The positive control (tin-impregnated PVC disks) was cytotoxic (grade 3) to L929 cells under the test conditions.

LDH staining of pretreated cultured human skin explants did not reveal toxicity of the mucopolysaccharides derived from psyllium husk.

Allergenicity. No positive skin response was obtained for any of the test or control guinea pigs. Under the conditions of this test, psyllium husk was therefore classified as having weak immunogenic potential (the lowest possible classification). This applies to extracts from the polypropylene sachet as well as the hydrocolloid sheet containing the psyllium husk granulate.

In the skin explant method, Langerhans’ cell migration from the epidermis was negligible (Fig. 8). Furthermore, the culture supernatants produced a slight but insignificant increase in interleukin-1β secretion by the explants. This indicated that no macrophage activation took place, which was apparent because of the slight increase in macrophage HLA-DR expression.
Discussion

We studied the characteristics of the mucopolysaccharide granulate derived from psyllium husk in Askina®Cavity. and Askina®Hydro related to fluid absorption (bacterial adherence, biocompatibility, macrophage stimulation, irritancy response and allergenicity).

Whereas Askina®Cavity is indicated for deep, chronic exuding and contaminated wounds with the aim of cleaning the wounds and stimulating granulation tissue formation, Askina®Hydro is intended for superficial, partial-to-full thickness, moderately exuding chronic or acute (surgical) wounds such as granulating venous leg ulcers, incisions, biopsy wounds, split skin donor sites, dermabrasions, etc. It is also indicated for superficial, slightly or moderately exuding and contaminated wounds. Before any wound repair can take place, the wound should be pathogen free. Psyllium has the unique property of selectively binding a host of bacteria through its specific polysaccharide composition, which behaves as ligands to bacterial receptors on the bacterial cell wall. Normally, bacteria use these receptors to adhere to the tissues (cells or matrix molecules). Through this binding they can inflict their pathologi-}

cal activity. Binding with the sugar moiety of psyllium is a decoy mechanism, rendering the bacteria impotent. The sustained and long-acting adherence and fluid absorbing property of psyllium in Askina®Cavity and Askina®Hydro make their use very practical.

Wound healing depends on three major principles:

i) Protection against external pathogens and irritants. Thus, a dressing should have a top layer that does not permit the entry of microbes, particulate material or solvents. The semipermeable top layer of Askina®Hydro fulfills these requirements. Furthermore, the dressing should stick to the skin so that there are no openings to the outside world, leading to further contamination. This is only possible when the dressing has the right adhesive properties, as well as the ability to absorb excess fluid. This was amply proven in this study.

ii) Maintenance of optimal moist wound environment. The unique property of absorbing fluid (exudate) and the creation of a stable gel without the wound drying out is provided by the combination of psyllium in the hydrocolloid and the semipermeable top layer.

iii) Optimal exchange of nutrients, growth factors, cytokines and waste products and toxins. If no nutrients, growth factors, or cytokines are added to the dressing, the only role they can play is to stimulate cells involved in the repair process. Psyllium stimulates macrophage functions. Macrophages play a pivotal role in wound healing: firstly in phagocytosis of tissue debris, then in release of building blocks, and finally, in the secretion of growth factors and cytokines necessary for wound repair and remodeling.

The wound dressing itself should also be wound friendly. It should not be painful while applied to the wound, which is sometimes experienced when the absorption power is too high. The dressing should not be irritating or toxic in itself. Psyllium appears to be easily biodegradable. This study demonstrates that the dressing and its components were neither toxic nor sensitizing. At removal or when being
changed, the dressing should neither damage the
delicate newly formed wound tissue nor the sur-
rounding skin with its protective horny layer. This in
itself can give rise to new defects. Removal of the
dressing should also be easy and pain-free.

In conclusion, these in vitro results confirm the cli-
cial data and demonstrate that Askina®Hydro and
Askina®Cavity are modern wound healing products,
which can play a role in all the above factors.

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