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Original article

Laboratory assessment of the efficiency of encasing materials against house dust mites and their allergens

Background: The current recommendation to reduce mite allergen exposure for mite-sensitive individuals is to use allergen-impermeable bed coverings. As these covers are made of various kinds of materials, they vary in quality. The objective of this study was to investigate the efficiency of different covering materials against house dust mites and their allergens *in vitro*.
Methods: Four types of materials including (1) plastic cover, (2) polyurethane-coated cover, (3) non-woven covers, (4) tightly woven microfiber covers and a regular cotton bed sheet (as a control) were evaluated using three methods: (i) heat escape method, (ii) Siriraj chamber method and stereomicroscopy, scanning electron microscopy and (iii) enzyme-linked immunosorbent assay (ELISA).
Results: We found that there was a statistically significant difference in allergen permeability among four types of coverings ($P < 0.001$). In terms of the impermeability to mites and their allergens, plastic- and polyurethane-coated covers were observed to be the best, followed by non-woven, woven and cotton-based bed sheets. A regular cotton-based bed sheet allows a significant amount of leakage of mite allergens. Both woven and non-woven material are efficient barriers against mite allergen in terms of impermeability. However, with regard to mite colonization, non-woven covers have the drawback of mites being able to penetrate and colonize within the fabric fibers. Woven covers are therefore recommended because of their major advantages of not allowing the colonization of mites within the fabric, being easy to clean, and comfortable.
Conclusion: The three assessment methods used in this study could be useful as a primary approach to evaluate the quality of covering materials *in vitro* using both pore size and ability to be colonized by mites on the materials as the key factors.

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Exposure to house dust mite (HDM) is an established risk factor for exacerbation of asthma. It has been demonstrated that high level of exposure to HDM is associated with more severe asthma (1, 2). Mites are found in many different sites in houses with the bedroom being the most prominent site (3, 4). Mattresses and pillows are the major habitats for dust mites (5). Several strategies have been used in an attempt to control house dust mite, including killing mites by physical or chemical methods. However, the most practical method recommended is to encase the bedding with an allergen-impermeable cover (6), which is considered the first approach in allergen avoidance (7). For example, in the Netherlands, the use of anti-allergic encasing has become a standard procedure in the treatment of HDM-allergic asthmatic patients (8). The main purpose of encasement is to block the leakage of dust mites and their fecal pellets from the bedding. Most of the previous studies of allergen avoidance using impermeable covers have emphasized the clinical benefit in asthmatic patients and demonstrated a reduction in mite allergen concentration after encasing the mattresses for a period of time (9–12). Although there are several encasing materials commercially available, the issue of quality in terms of house dust mite protection is often overlooked. Methods to evaluate impermeability are seldom mentioned. Vaughan et al. (13) studied the permeability of woven and non-woven fabrics by measuring the pressure gradient across the fabric. The results indicated that both types of fabrics can be permeable to air and still provide efficient barriers to cat and dust mite allergens. It was recommended that fabrics with a pore size of 2–10 μm are suitable for use as encasing materials because of their ability to block the passage of all dust mite allergens (13). The objective of this study was to investigate the efficiency of different cover materials against house dust mites and their allergens *in vitro*.

Materials and methods

Bed-cover materials

Four types of materials claimed as 'mite-proof' or 'anti-mite' covers, i.e. (1) a plastic sheet cover (brand A), (2) a polyurethane coated cover (brand B), (3) non-woven covers (brands C and D), (4) tightly woven microfiber covers (brands E, F and G) and a regular cotton bed sheet (H) were compared in terms of actual mite and allergen impermeability. The observers were blinded to the type of cover.

Methods

The methods used in this study were: (1) the heat escape method, (2) Siriraj chamber method, stereomicroscopy and scanning electron microscopy (SEM) and (3) enzyme-linked immunosorbent assay (ELISA) for Der p 1 (the major group 1 allergen from the dust mite *Dermatophagoides pteronyssinus*) (14).

Heat escape method. Each material was first stretched over the top of a beaker. Ten mites obtained from culture were then placed on the surface of the cover and were forced to move downward by heat from a 100-W light bulb. Mite behavior was observed under a stereomicroscope. If the encasing materials were porous, mites would easily pass through under this stimulus.

Siriraj chamber method. To determine the ability of different encasing materials to block the actual mites, special apparatus to keep mites on encasing material is needed. A Siriraj chamber (unpubl. data), a recently developed apparatus to assess the effectiveness of anti-mite agents and to study house dust mite biology was used. This apparatus is able to restrain mites and other small living insects. It consists of a $5 \times 5 \times 3$-cm acrylic box with a $4.5 \times 4.5 \times 0.3$-cm plastic sheet inserted at the top with a 1-cm diameter aperture in the middle for ventilation. The hole was first covered by a 2×2-cm piece of the encasing material being evaluated, followed by an acrylic ring. About 10–20 adult mites were placed in the middle of the ring, covered by the chamber lid and locked to prevent mite escape. The chamber was maintained at 25°C and 75% relative humidity for 1 week prior to opening and inspection of the location of mites by stereomicroscopy and SEM. Photomicrographs were taken to demonstrate the texture of cover material, and the presence of mite droppings, and mites in fabric fibers.

Mite allergen detection. Dust was collected from vacuum cleaner bags and then homogenized by sieving three times.

Three grams of dust was then kept between two layers of the same kind of encasing material being tested. After vacuuming for 2 min by a vacuum cleaner with dust trap (Alk, Hørsholm, Denmark), the amount of dust retrieved and the concentration of mite allergen, Der p 1 were determined using a two-site monoclonal-based ELISA (Indoor Biotechnologies, Charlottesville, VA, USA). The percentage of allergen impermeability was calculated as shown below.

$$\%\ \text{impermeability} = \frac{\text{Total amount of Der p1 in the dust recovered from the encasing}}{\text{Total amount of Der p1 in the dust added to the encasing}} \times 100$$

The total amount of Der p 1 (μg) can be obtained by multiplying the allergen concentration (μg/g of dust) by total amount of dust (g).

Statistical analysis

To compare the percentage of allergen impermeability from the eight different brands and four different types of mattress covers, Kruskal–Wallis one-way analysis of variance by rank was employed using a two-sided type I error of 0.05. If a statistically significant difference was observed, pairwise comparison was then performed using Bonferroni's method to control for overall type I error. All statistical analyses were performed using SPSS/PC Version 10.0. The number of replicates used in each arm of the experimental design was seven.

Results

The heat escape method revealed that all eight encasing materials tested, except the regular cotton bed sheet were able to block the mite.

Figure 1 shows the different weaving pattern of the two brands of non-woven material. Mites could easily penetrate both fabrics and colonize them within a few days. Figure 2 illustrates the tightness of the weave and the pore size in a woven cover relative to mite body and fecal pellet size.

Immunoassay results show that there is a statistically significant difference in impermeability among the eight brands of encasing materials evaluated ($P < 0.001$, Fig. 3). Plastic and polyurethane covers were the most impermeable to dust mites and their allergens, whereas the regular bed sheet was the worst. Non-woven cover demonstrated better impermeability against mite allergen than woven covers, but mites could penetrate and survive within the fabrics as shown in Fig. 1. When encasing materials were categorized into four major types, i.e. (1) plastic and polyurethane, (2) non-woven, (3) woven and (4) cotton-based, a statistically significant difference in percent impermeability was also observed ($P < 0.001$). Significant differences were also observed between all possible six pairwise comparisons.

Discussion

The main purpose of this study was to investigate the efficacy of encasing materials against dust mites and their allergens using three methods: (1) the heat escape method, (2) the Siriraj chamber method, and (3) ELISA.

The heat escape method has an advantage of simplicity. If any encasing material allows a mite to go through, it will also allow allergens to pass through (average mite body size 300 μm compared with a fecal pellet of 10–40 μm) (15). Thus, there is no need for further investigation of allergen impermeability for that material. For the Siriraj chamber method, the mite specimen has to be prepared using a Siriraj chamber before microscopy can be performed which has the advantage of being able to restrain mites in the encasing material under evaluation. By using SEM, the texture of the material or the

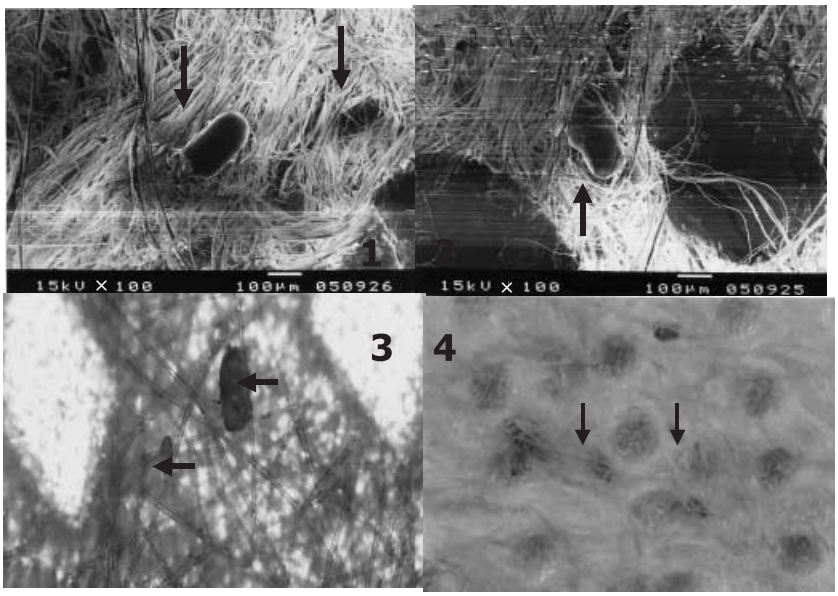

Figure 1. Photomicrographs of mites on non-woven covers. Brand C (1, 2): SEM pictures demonstrating mites penetrating into the fabric fibers. Brand D (3): light microscope picture showing mite egg and mite body on the fabric fibers. Brand C (4): light microscope picture demonstrating mites colonized beneath fabric fibers.

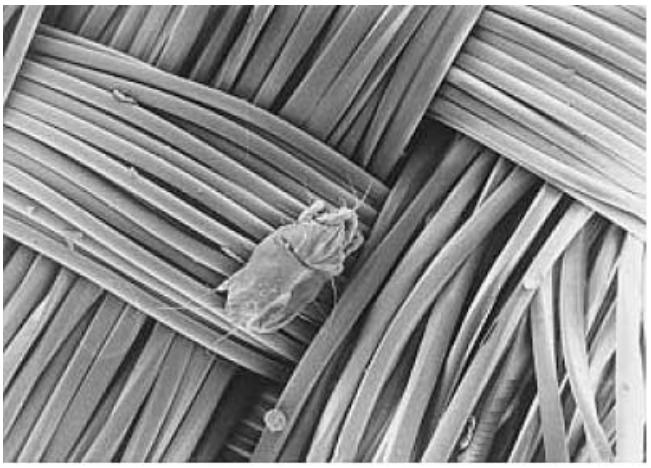

Figure 2. Photomicrograph of the texture of a tightly woven microfiber cover (E) showing the relative pore size of the material, mite body size and a fecal pellet.

colonization of mites in fabrics could be observed. An immunoassay ELISA was applied to determine the allergen concentration in dust passing through the fabrics and Der p 1 was used as a marker allergen in dust. These three methods have two major advantages. First, they can demonstrate both the pore size and colonization of the materials by mites. Secondly, they are actually simple routine laboratory techniques and are useful as a primary approach to evaluate the quality of mite-proof covers *in vitro*. Vaughan et al. (13) developed a method for testing encasement materials by measuring the pressure gradient across the fabrics of woven and non-woven

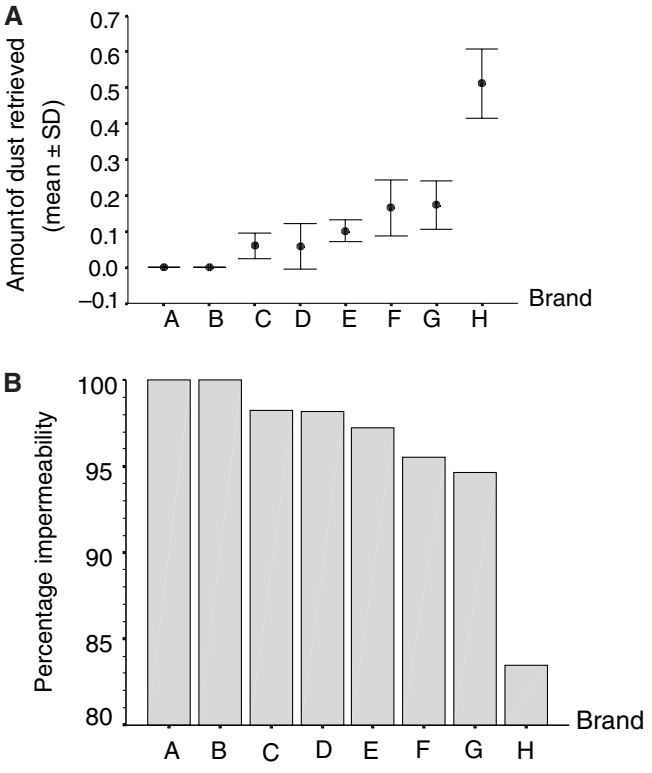

Figure 3. Comparison of the amount of dust retrieved (A) and the percentage of impermeability to Der p 1 allergen (B) between eight brands of encasing materials; A, plastic; B, polyurethane; C, D, non-woven; E, F, G, woven; H, cotton bed sheet.

covers. They suggested that pore size of woven fabrics was a key factor in blocking allergens and also recommended the fabrics of 2–10 μm (approximately 6 μm) in pore size will allow air flow but completely prevent the passage of allergen. In our study, we found that not only the pore size, but also the texture of fabrics should be considered. To compare the efficacy of different encasing materials, the ratio of the total amount of mite allergen retrieved from dust to the initial fixed amount of mite allergen was defined as percent impermeability. The total amount of mite allergen retrieved from dust was much more important than the total amount of dust retrieved. This is due to the fact that very high mite allergen concentration could be derived from a small amount of dust retrieved (Fig. 3).

This *in vitro* study showed that four types of encasings used for mattresses ranked in impermeability as follows: plastic, polyurethane, non-woven and woven. Although encasings coated with an impermeable layer enabled no penetration by allergen, anecdotally they are also reputed to be the least comfortable. We think that an ideal encasing material used for bedding encasing should have two important properties; first, to be able to block the passage of both mite bodies and faecal pellets, and secondly, to be comfortable. Non-woven encasings showed significantly less permeability than woven encasings, and were also shown to provide a habitat for colonization by mites due to its compressed fiber structure (Fig. 1). Tightly woven microfiber cover could significantly reduce mite allergens compared with a regular cotton bed sheet which allow a significant amount of leakage of mite allergens through the material. This is due to the fact that a regular cotton bed sheet has a relatively large pore size (400–500 μm) which is significantly larger than the size of the average adult mite body. Previous studies have also reported that unencased mattresses could achieve high mite allergen levels within 4 months of use (5).

With regard to washability, most non-woven encasing covers claim to be water-resistant and washing is not recommended. As mite allergens are quite soluble in water (16), any washing method can remove most of the dust and allergen (17). Therefore, washable covers are superior to non-washable covers.

This study confirms the benefit of encasement to avoid house dust mite exposure *in vitro* and points out the practical differences between woven and non-woven covers, based on their ability to be colonized by mites. The quality of the material with regard to pore size and ability to be colonized by mites should also be considered.

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