Mite penetration of different types of material claimed as mite proof by the Siriraj chamber method

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Background: There are different materials and principles used in the construction of bed encasings. Although these covers claim to have antimite properties, they might not be mite proof.
Objective: This study evaluated the effectiveness of mite penetration of these covers by using the Siriraj chamber method.
Methods: Thirty-two covers collected from 9 different countries were categorized according to the materials used to manufacture them. They were (1) tightly woven, (2) film or membrane coated and loosely woven, (3) acaricidal coated and loosely woven, (4) nonwoven, (5) film coated and nonwoven, (6) acaricidal coated and nonwoven, and (7) plastic. Adult mites, *Dermatophagoides pteronyssinus*, were placed on either the outer or inner surfaces of each of the test fabrics for 3 replications, resulting in a total of 6 samples per fabric. All samples were observed for penetration every day for 1 week under a stereomicroscope. If a single mite penetrated any fabric, it was scored as a penetration.
Results: Mites penetrated (1) into all samples of film-coated woven and nonwoven covers, an acaricide-coated nonwoven cover, and nonwoven types; (2) from both sides and colonized within the matrix of some samples; and (3) completely in other cases. All of the woven covers and the plastic cover prevented mite penetration. Photomicrographs documented all penetrations.
Conclusions: Tightly woven covers and plastic prevent mite penetration, whereas nonwoven, loosely woven, acaricide-coated, and laminated materials do not. The Siriraj chamber method adequately evaluates the effectiveness of antimite barriers.
Clinical implications: For mite avoidance, allergists should recommend the use of tightly woven covers on suspected bedding containing dust mites. (J Allergy Clin Immunol 2006;118:1164-8.)

Key words: *Mite-proof covers, antimite covers, encasement of beddings*

The use of encasings on bedding is widely advocated by both asthma management guidelines and by allergists to reduce exposure in beds to the allergens produced by house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*).[1-4] Such encasings form a physical barrier around items of bedding, which prevents the movement of mites between different parts of the bed, thus limiting colonization. They also prevent the allergens from these encased reservoirs from becoming airborne, thereby reducing allergen exposure. Although regular laundry can additionally be used to remove allergens and, to some extent, mites from washable items of bedding,[5] the washing of mattress encasings is seldom feasible because they are too cumbersome to regularly remove, launder, and refit. At best, encasings can be wiped down, and this is suggested in some instructions to users.

The strong global advocacy of encasings has led to the development of many different types of encasings around the world. These differ widely in the type of materials used for their construction, which in turn affects their permeability to allergens and humidity. However, there are no guidelines as to the desirable properties for such encasings and few studies of their different performances. One of the few comparative studies showed that a fabric pore size of 10 μm was sufficient to prevent the passage of mite allergens under the conditions of testing by using a modified Fussnecker chamber, whereas a pore size of 2 μm was required to prevent the passage of the smaller particles carrying cat allergens.[6,7] Several other studies have shown large differences in the permeability of different types of encasings to water vapor, which probably affects their comfort when used.[8,9] At this time, the ideal characteristics for encasings were low passage of allergens and high passage of water vapor.

Three years ago, Mahakittikun et al[10,11] showed that there was another distinguishing and potentially important feature of encasings. This was that some encasings supported the colonization of live mites within the nonwoven

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fibers of the fabric structure, and this occurred even though these encasings functioned adequately as barriers to the movement of allergens through the fabrics. As a consequence, these encasings, which were unlikely to be washed, could function as a primary source of live mites and of allergens, even if the other bedding was washed or reservoirs were isolated. These studies were performed by using a Siriraj chamber, which in effect isolates cultured mites on a fabric surface and enables the observation of their passage into or through the fabric sample undergoing testing.

The original study was performed on a few samples that were locally available. The current study extends these findings to include a much wider range of samples of fabric used for encasing bedding that have been collected from 9 countries to determine which of the different types of fabrics in use will permit colonization and to suggest that this is another property of fabrics that should be considered when choosing the optimal encasing for use.

METHODS

Fabric samples and subjects

Thirty-two pillow encasings claiming to provide protection from mites and their allergens were collected from 9 countries: Australia (n = 6), Canada (n = 1), France (n = 1), Italy (n = 1), Germany (n = 1), Japan (n = 4), the United States (n = 12), England (n = 1), and Thailand (n = 5). All covers were obtained or bought locally from shops in that country, although many had been manufactured elsewhere. The samples were classified into 7 categories based on the materials used to manufacture them as follows: tightly woven (n = 16), film or membrane coated and loosely woven (n = 4), acaricide coated and loosely woven (n = 1), nonwoven (n = 7), film coated and nonwoven (n = 2), acaricide coated and nonwoven (n = 1), and plastic (n = 1). The samples were cut into 2 × 2–cm squares for Siriraj chamber testing. The outer and inner surfaces of each fabric were then exposed to house dust mites. Additionally, 2 brands of nonwoven covers, which had been in normal domestic use by one of the researchers for 4 months, were included to enable comparison of fabric change.

House dust mites

The mites used were a strain of *D pteronyssinus* maintained in the laboratory of the Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand. The mites were separated from their culture media by using a specially designed mite isolation apparatus, which used a heat-escape method to force the mites down and through a sieve away from the food source. Ten adult mites of either sex were selected by using a stereomicroscope and transferred to each fabric sample.

Siriraj chamber method

The Siriraj chamber[12] consisted of a 5 × 5 × 3–cm acrylic box with a 4.5 × 4.5 × 0.3–cm plastic sheet inserted at the top and 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. After mite placement within the acrylic ring, the lid was closed and locked to prevent the mites from escaping. This apparatus effectively localized and restricted the mites to the test samples.

Penetration of mites into fabric samples

Briefly, mites were placed separately on the outer (the surface facing away from the inside of the pillow) and inner (the closest to the pillow filling) surfaces of each test fabric. Each test was conducted 3 times for each side of the 32 samples. At any one time, a minimum of 6 chambers was used. The chambers were heated with 100-W light bulbs positioned 10 cm above the closed lid for 15 minutes to force the mites to attempt to penetrate into the fabric.

This procedure, which was done only once at the beginning of testing, was not sufficiently rigorous to cause the mites to observably dehydrate and become inactive. Testing was done on an open workbench at room temperature (23°C ± 2°C) and at 75% relative humidity. The culture of mites in the chamber was continued for 1 week, with observations of the mites for penetration into the matrix of the fabric each day. The mites were not fed while in the chamber. Penetration was defined as infiltrating just below the surface or going directly into the matrix of the fabric. Even if only 1 of 10 mites penetrated at any time during the week, that fabric was scored-categorized as conducive to being penetrated. The frequency count of the penetrated and nonpenetrated fabrics was then converted to percentages of the total number of fabrics (n = 32) tested. The location of penetration, whether it was from the outer surface to the inner surface, vice versa, or both was similarly scored. Representative samples of photomicrographs depicting penetration, colonization, or both, which was defined as a group of mites living together, including mating and producing eggs and offspring, were also taken with either the stereomicroscope or with a cryo-scanning electron microscope.

TABLE I. The number and total percentages of samples showing mite penetration

Category	No penetration	Some penetration
1. Tightly woven	16	0
2. Film coated and loosely woven	0	4
3. Acaricide coated and loosely woven	0	1
4. Nonwoven	0	7
5. Film coated and nonwoven	0	2
6. Acaricide coated and nonwoven	0	1
7. Plastic	1	0
Totals	17 (53%)	15 (47%)

RESULTS

The percentage of mites penetrating on any surface of the fabrics is shown in Table I. Fifty-three percent of the covers showed no penetration, whereas approximately 47% showed some degree of penetration. All 16 of the tightly woven covers and the 1 plastic cover did not allow any penetration, whereas all of the nonwoven covers, whether they were film coated or acaricide coated, allowed penetration. The location of penetration for the 15 covers shown in Table I is presented in Table II. Nine (60%) of 15 covers allowed penetration from the outer surface only, whereas only the inner surface did not permit any penetrations. Bidirectional mite infiltration was observed for 4 of the nonwoven covers, 1 acaricide-coated and nonwoven cover, and 1 acaricide-coated and loosely woven cover (40%).

Fig 1 shows 4 pictures of mites embedded on 4 fabric samples; 3 were taken with a scanning electron microscope (Fig 1, *A*, *B*, and *D*) and one with a stereomicroscope (Fig 1, *C*).

TABLE II. The number and percentages of mite penetration in different locations for the various types of loosely woven and nonwoven covers

Category	Mite penetration		
	Outer surface only	Inner surface only	Both outer and inner surfaces
1. Tightly woven	0	0	0
2. Film coated and loosely woven	4	0	0
3. Acaricide coated and loosely woven	0	0	1
4. Nonwoven	3	0	4
5. Film coated and nonwoven	2	0	0
6. Acaricide coated and nonwoven	0	0	1
7. Plastic	0	0	0
Totals	9 (60%)	0	6 (40%)

Fig 1, *A*, depicts a ventral view of a male mite (indicated by *arrow a*) localized between the loosely woven fibers of a laminated cover. Clearly visible are the 2 rounded anal suckers to the left of the arrow and the fourth pair of legs to the right of the arrow overlapping the body. The laminate floor is indicated by *arrow b* adjacent and to the right of the mite. The mite inserted itself through the fabric's hole to reside on the floor of the basement membrane beneath the loosely woven fibers.

Fig 1, *B*, depicts the exoskeletal remains of a mite that shed its integument among the disorganized fibers of a nonwoven fabric. The presence of skin debris and molting is a sign predating subsequent developmental stages and possibly colonization within the matrix of the material.

Fig 1, *C*, is a photomicrograph taken with a stereomicroscope showing 7 dead mites localized on an acaricide-coated nonwoven cover. The presence of dead mites was indicated by a change in body color (from white to brown), shrinkage of the body caused by dehydration, withdrawal of the legs close to the body, and a lack of mobility. It is not immediately evident whether any of the mites penetrated beneath the surface, although Table II indicates that for this particular cover, mites penetrated from both the inner and outer surfaces.

Fig 1, *D*, shows 5 mites *(arrows)* that have penetrated the unorganized fibers of a nonwoven cover. The presence of so many mites in close proximity might be construed as another indication of colonization.

Fig 2 shows 2 brands of nonwoven covers before and after 4 months of use. The new unused covers (Fig 2, *A* and *C*) have only minimally loose fibers. This contrasts sharply with the unraveled, separated, and tangled threads of the used fabrics seen in Fig 2, *B* and *D*. This provides an easy access for mites to readily penetrate into the substrate of the material.

Fig 3 presents a view of a tightly woven fabric with systematized regular fibers allowing little space for penetration. This micrograph can be compared with the loosely woven fibers shown in Fig 1, *A*, which shows mite infiltration.

DISCUSSION

Even though mite-proof covers are widely recommended for use by patients with mite allergy to avoid allergen exposure, there is little published research evaluating aspects of their performance and the differences between the available types of covers. The main purpose of so-called antimite bedding encasement is to provide a barrier to the movement of allergens and live mites through layers of bedding. Thus it serves as a protection from exposure to dust mite allergens contained within mite fecal droppings[13] by providing a barrier to the movement of live mites, which limits defecation and the spread of mite fecal pellets associated with colonization. The ideal mite cover should have at least 2 important characteristics. The material used in its construction should (1) block the leakage of mite allergens from the inside of the bedding and (2) prevent mites from penetrating through the covers in either direction. An additional virtue is the movement of water vapor, which reduces the feeling of sweating and discomfort that has been associated with some encasings.[8] Barriers vary in the types of material used in their construction, which in turn reflect different principles used to reduce the presence and movement of mites and their aeroallergens. This study categorized the covers into 2 groups: woven or nonwoven in construction. The former consisted of interwoven directional cotton or synthetic fibers, whereas in the latter the fibers were synthetic and oriented randomly and in layers. Furthermore, to increase the impermeability, some fabrics had bonded layers of a film coating the inner side of the cloth. Another variation of different types of materials is the incorporation of a contact acaricide to kill live mites.

The characteristics of these fabrics determine their effectiveness as barriers, as well as comfort. In cases in which woven or nonwoven fabrics have pores, it had previously been shown that the pore size controls the leakage of mite allergen, with pores of between 2 and 10 μm (average, 6 μm) blocking most mite allergens.[6,7] Plastic, which is pore free, is the best barrier in terms of blocking. However, it is also the least comfortable because of zero ventilation and over time has the tendency to become a haven for mold spores.[7] For acaricide-coated materials, the pore size is usually not a major factor in blocking mites and their allergens because the mites are eradicated on contact with the chemicals. However, such fabrics might still allow the passage of allergens. This study showed that the single plastic cover and all the tightly woven covers did not allow the penetration of live mites from either side. Of the 16 woven covers in this category, 9 were from the United States, 3 were from Japan, 2 were from Thailand, 1 was from Germany, and 1 was from France. Fig 3 is a representative illustration of a tightly woven fabric randomly sampled from the 16 impenetrable covers of this study. Its patterning is highly structured, systematized, and quite regular, with

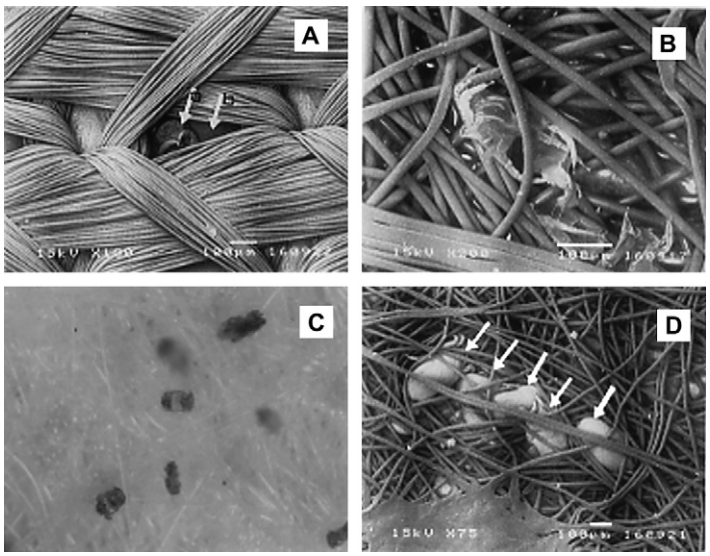

FIG 1. Pictures of mites within the fibers of a laminate-coated and loosely woven cover (**A**; ×100 magnification, scanning electron microscope), evidence of molting among nonwoven fibers (**B**; ×200 magnification, scanning electron microscope), dead mites on an acaricide-coated and nonwoven cover (**C**; ×40 magnification, stereomicroscope), and a group of mites in the matrix of a nonwoven cover (**D**; ×75 magnification, scanning electron microscope).

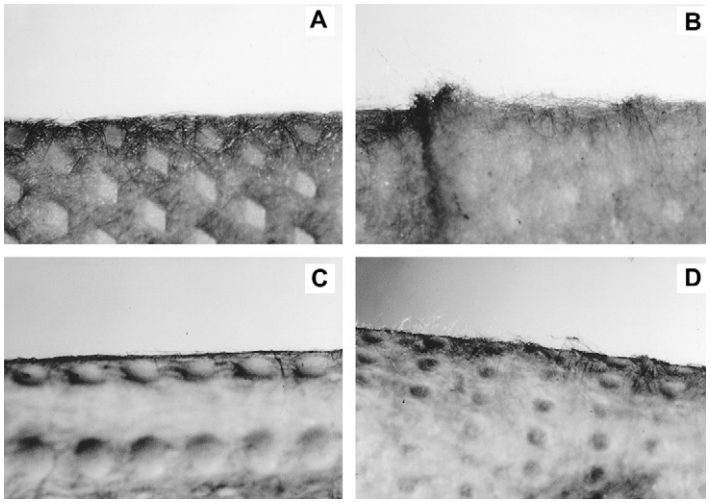

FIG 2. Stereomicrographs of 2 brands of nonwoven fabrics before (**A** and **C**) and after (**B** and **D**) 4 months of use (×40 magnification).

little space for mites to infiltrate. Technically, the construction of such fabrics is described as triaxial or biaxial,[14] depending on the pattern of weaving, and all involve sets of yarns or twisted fibers tightly interlaced at right angles to each other, allowing no penetration of mites but some diffusion of water vapor.

All types of nonwoven (chemically coated, laminated, film coated, or nonwoven alone) covers might be penetrable by mites. Such nonwoven webs are made from fibers that have been randomly tangled, fused, glued, or melted together, resulting in an unorganized and nonstructured disarray. Pore size within the substrate of the fabric is not an important consideration in construction. There are some brands, however, that claim to be constructed of multiple layers, with a coarse outer layer covering an inner layer of finer structures that prevent the complete passage of mites and allergens from one side of the cover to the other side. All 7 of the nonwoven covers were observed to be penetrated by dust mites. Moreover, evidence of a mite colonization was suggested in 2 of the electron micrographs of the nonwoven covers, as shown in Fig 1. As a caveat, however, it should be mentioned that 7 nonwoven covers is a relatively small number and might not be representative of the larger class of nonwoven fabrics. There might indeed be some types of nonwoven fabrics (non–spun–bonded as wet-laid fiber webs or melt-blown fiber webs),[14] which might be impervious to mites, but this has not been explored. Clearly, additional research is

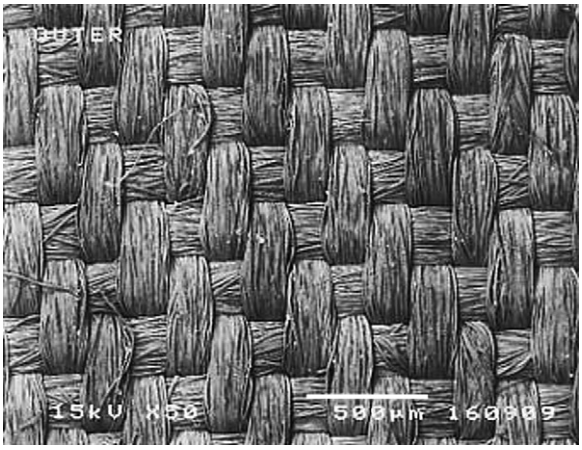

FIG 3. View of a tightly woven fabric with systematized regular fibers.

needed to clarify the type and extent of penetration of mites into nonwoven fabrics and the likelihood that such penetration results in persistent colonization of the fabrics. A total of 8 film-coated and acaricide-coated loosely woven and nonwoven fabrics did allow mite access. None of these should be used as antimite barriers because although they might prevent penetration of mites through the fabrics, they allow colonization. Such colonization would not be prevented by the wiping down of fabrics, as recommended in some maintenance instructions. These results address the penetration by dust mites of the surface of fabrics used in pillow encasings and sold to provide protection against these mites. It was evident from this study that the nonwoven fabrics will allow surface penetration, and in some cases they appeared to allow colonization. Additionally, it was observed that normal use of fabrics made this more likely, as shown in Fig 2. Although clinical recommendations for use of encasings remains controversial,[15-17] at least in terms of primary prevention of allergies, it is clear that the materials used to construct covers have not been adequately considered, and the design in some cases remains suboptimal. Ideally, such covers should both provide comfort by allowing transfer of air and moisture, as well as preventing the passage of allergens and the colonization by mites.

In this regard the Siriraj chamber was used as a tool for observing mite penetration and acaricidal activity. Consequently, it provides a simple method to evaluate encasings to understand their interaction with live mites. However, the use of this chamber cannot measure the allergens in mite feces, but in all or mostly all of the photomicrographs taken, wherever there were mites, fecal matter was observed but not enumerated. In a subsequent study, which we have just completed, using the heat-escape method, ELISA for measuring allergen content, and an analysis of the physical characteristics of more than 50 fabrics, we are attempting to (1) establish criteria for the materials used in the construction of antimite covers and (2) explore the relationship between the physical characteristic of the fabrics and their protective ability for both live mites and their allergens.

Within the limitations of this experiment, it can be concluded that among the 7 categories of available antimite materials, tightly woven covers provide an effective barrier against mite penetration. Nonwoven, loosely woven, acaricide-coated, and film-coated covers do not prevent mite penetration. The Siriraj chamber method is an adequate procedure for evaluating the effectiveness of antimite barriers, whether the mechanism is blocking or acaricidal. Further study should explore the protective ability of antimite covers in terms of their ability to block allergens with a view toward establishing guidelines for consumers to choose materials that exhibit antimite properties.

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