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A New Approach to Assess the Retention of Allergen (Der *f* 1 and Der *p* 1) on Anionic and Nonanionic Cotton Bedding Sheets After a Vigorous Sequential Cleaning Regime as Assessed by ELISA

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ABSTRACT: Anionic surfaces can interact with a variety of biological molecules and when used on bedding material may have the potential of binding with and sequestering protein allergens such as those produced by dust mites. However, no method exists to evaluate the efficacy of this claim. The purpose of this study was to develop a method using enzyme-linked immunosorbent assay (ELISA) to evaluate the capability of anionic treated cotton bedding sheet material to retain medically important dust mite allergens in direct comparison with nonanionic cotton bedding sheet material from a single manufacturer. Anionic, and nonanionic cotton bedding sheets were challenged singularly with water suspended pulverized dust mite *Dermatophagoides farinae*, water suspended pulverized dust mite *D. pteronyssinus*, or water alone as a negative control. All dried inoculums were subjected to four sequential extraction series using water in the first two separate extractions and water with surfactant in the last two separate extractions. All four extractions were assessed by ELISA using monoclonal antibodies specific for medically important protein allergens Der *f* 1 (*D. farinae*) or Der *p* 1 (*D. pteronyssinus*), respectively. There were a total of 25 replicates for each assessment. Our findings demonstrate that nearly all of the Der *f* 1 and Der *p* 1 was released from the nonanionic fabric after the first water extraction. Conversely, most of the Der *f* 1 and Der *p* 1 allergens were retained by the anionic fabric after both water extractions and was released only after surfactant was used. Our data indicated that > 80% of Der *f* 1 and Der *p* 1 was released from the

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nonanionic fabric after the initial two water extractions while 80% of Der *f* 1 and Der *p* 1 was retained by anionic fabric after the same extractions. The method proved to be robust and data was reproducible. Protein extraction using less stringent to high stringent conditions demonstrated the effectiveness of anionic fabric in retaining dust mite proteins that cause allergy. This method can be used to assess other materials while allergists should consider the use of anionic bedding as it effectively retained dust mite allergens Der *f* 1 and Der *p* 1 until laundering can remove them from the household.

KEY WORDS: dust mites, Der *f* 1, Der *p* 1, cotton bedding sheet, anionic, ELISA.

INTRODUCTION

ALLERGY CAN BE described as increased sensitivity to certain foreign allergen accompanied by sneezing, itching, watery eyes, difficulty breathing, and rash. Severe allergenic reactions, although rare, can even be fatal. There are many forms of allergens produced from a variety of animals, insects, and plants, but a major source of allergy in the indoor environment is caused by dust mites. A recent study, identified dust mite allergy as the most prevalent of allergic diseases, affecting up to 40 million people in the United States, at a cost of approximately \$1 billion annually on medications and preventive measures [1]. The most thoroughly studied dust mite allergens that occur indoors are group 1 allergens Der *f* 1 and Der *p* 1 derived from *D. farinae* and *D. pteronyssinus*, respectively [2]. It appears that exposure to dust mites and allergen mainly occurs from bedding although dust mites have been found in carpets and upholstered furniture [3–9]. There is evidence that pre-existing conditions like asthma increase the susceptibility of allergic response to dust mite allergen with greater severity of disease and increased use of healthcare [3,4,10].

There have been many studies evaluating materials, activities, and strategies specifically designed to reduce dust mite allergen exposure to susceptible patients by: (1) removing materials contaminated with dust mites, (2) pre-treating materials with insecticide to kill dust mites, (3) developing barrier materials that limit dust mite movement [5,11–18]. However, there are disadvantages associated with each of these approaches. Dust mite allergen on bedding material can be aerosolized by removing and laundering of the materials, while the use of insecticide is unfavorable because it will not reduce allergen and washing will remove it over time. Barrier sheeting that limits water vapor permeability create conditions that promote the growth of other undesirable allergen

producers such as mold. In summary, none of these approaches will remove the actual source of allergy, dust mite allergen. Any technology that offers an alternative approach to reduce exposure to dust mite allergens would be advantageous.

Anionic (negatively charged) surfaces have been developed for a variety of purposes. Some interesting characteristic of anionic surfaces is their capacity for binding biological materials by inducing conformational changes making certain proteins more susceptible to cleavage by a variety of proteases, their ability for membrane binding of bacteria and damaged apoptotic human cells, and their potential use as a microbicide [19–23]. However, none of these studies investigated whether pure protein allergen can interact with anionic surfaces and whether the binding is rigorous.

The purpose of the present study was to evaluate the retention efficacy of both *Der f* 1 and *Der p* 1 allergens on anionic and nonanionic 100% cotton sheets after a vigorous sequential cleaning regime. None of the previous studies evaluated anionic coatings on materials such as bedding. As no existing method was appropriate for evaluating the retention of allergen on bedding materials, a new method needed to be developed.

METHODS AND MATERIALS

Bed sheets – The bed sheets RTI tested were composed of 100% cotton with a 300-thread count identified on the packaging as Intel-Tex intelligent anionic fabric (treated) and Hampton House Luxury Jacquard Hems (untreated). The bed sheets were provided to RTI in ‘for sale’ zippered final product packages. Southern Textile Research (STRA LLC, Esley, SC) applied the anionic ionized chemistry to the treated sheet by submersion in Ionized Wetter 102 and 105, Ionized Softener 350 and 360, Ionized Polymer 214, and Citric Acid (pH 4.0) (Piedmont Chemical Company, High Point, NC) in a proprietary manner.

Allergen – Lyophilized whole pulverized dust mite *D. farinae* and *D. pteronyssinus* were purchased from Greer (Lenoir, NC). Each was suspended separately in sterile distilled water (dH₂O) to 67 µg of total protein per milliliter.

Testing – The bed sheets were cut into manageable size (25 cm²) from overlapping sheeting and neatly stacked into treated (T) and untreated (U) piles. Test samples (1 cm²) were cut from these piles. Randomly selected samples were inoculated singularly with 10 µL of *Der f* 1 (F), *Der p* 1 (P), or water (W) as a negative control. There was a total of 25 TF, 25 TP, 10 TW, 25 UF, 25 UP, and 10 UW in total. The inocula were allowed to absorb onto the fabric for 5 minutes followed by drying under vacuum at 30°C for

30 minutes in a centrivap (Savant Instrument, Farmingdale, NY). Each test piece was placed in individual tubes and extracted using water or water with Tween-20 (0.05% v/v) surfactant in four sequential steps as follows. The first extraction was accomplished by adding sterile dH₂O to the each tube containing test sample. The tube was sealed and agitated for 30 seconds in a beater (Biospec Products, Bartlesville, OK) on mix mode. All liquid was decanted to a new tube labeled E1. A second aliquot of sterile dH₂O was added to the test sample, extracted as above, and decanted to a new tube labeled E2. The third extraction was in an aliquot of sterile dH₂O with Tween-20. The tube was agitated as described above and liquid decanted to a new tube labeled E3. The fourth and final extraction was in sterile dH₂O with Tween-20 as before and decanted to a new tube labeled E4. All extracts were either used immediately or stored at 4°C until needed without any loss of activity (data not shown). We used 10 µL of each 500 µL extraction in each 100 µL enzyme-linked immunosorbent assay (ELISA) reaction. ELISA was conducted as per manufacturer's recommendation's (INDOOR Biotechnologies Inc., Charlottesville, VA) with some modifications. Standards were used as manufacturer recommended and all incubations were done at 37°C. HPLC purified anti Der *f* 1 mAb 6A8 and anti Der *p* 1 mAb 10B9 stock solutions (2 mg/ml in PBS) were suspended separately in 50 mM carbonate-bicarbonate buffer (pH 9.6) and aliquoted into polystyrene microtiter plates (NUNC Maxisorp) at a concentration of 200 ng/well. The plates incubated for 1 hour. Plates were inverted and 'smacked out' onto a stack of paper towels followed by three washes of PBS-Tween-20 smacking out between each wash. The plates were blocked for 30 minutes with 0.1 mL 1% BSA PBS-Tween-20 and washed three times with PBS-Tween-20 as before. Extracted allergen was added and plate incubated for 1 hour. Plates were washed three times with PBS-Tween-20 as before and then incubated with 1/1000 dilution of biotinylated secondary antibody for 45 minutes. Plates were washed three times with PBS-Tween-20 as before and incubated with 0.1 mL 1/1000 dilution of Streptavidin-Peroxidase (Sigma, St Louis, MO) for 30 minutes. Plates were washed three times with PBS-Tween-20 as before and incubated with 1 mM ABTS in 70 mM citrate phosphate buffer (pH 4.2) and 1/1000 dilution of hydrogen peroxide until color change occurred (approximately 5 minutes). At this time absorbance was read at 405 nm in a Genios Multilabel Plate Reader (Tecan US, RTP, NC). Absorbance was monitored until values between 2.0 and 2.4 were obtained. The absorbance readings in this range are directly proportional to the quantity of allergen bound in the ELISA and values are interpolated from the respective standard and blank control curves.

RESULTS

A total of 60 each of T and U fabric swatches were analyzed. There were 25 replicates each coated with either Der *f* 1 or Der *p* 1 and 10 replicates each coated with sterile water (blank). ELISA kit standards (pure allergen) were included to verify the validity of the assay and do not directly relate to the quantity of allergen that bound to the material, but instead is intended to quantify the amount of allergen extracted from each fabric. As illustrated in the bar graphs (Figure 1) the majority of both Der *f* 1 (Figure 1(a)) and Der *p* 1 (Figure 1(b)) allergen extracted readily from the untreated fabric with a single water extraction (UFE1). After a second water extraction, almost all allergen was removed from the untreated fabric (UFE2). Conversely, the treated fabric retained more Der *f* 1 and Der *p* 1 allergen as only a very small fraction of each was removed after the same two water extractions (TFE1 and TFE2). The majority of both allergens were released only after the third extraction using water-surfactant (TFE3). After the final water-surfactant extraction almost all allergen is removed from the anionic treated fabric (TFE4) while background levels were observed for untreated fabric (UFE3 and UFE4).

The actual percentage of extractable allergen is presented in Figure 2. The stacked graphs represent 100% of the allergen that was released from either the treated or untreated fabric after the four extractions rather than the amount that was applied as some may have remained bound to the fabric despite repeated extraction. It is evident from the graphs that approximately 80% of the detectable Der *f* 1 (Figure 2(a)) and Der *p* 1 (Figure 2(b)) was released in the first two water extractions of the untreated fabric while 25% or less was similarly released from the treated fabric. In other words, approximately 78% of either allergen was retained until the third and fourth extractions by the treated fabric.

The total amount of allergen recovered from treated and untreated fabric was statistically indistinguishable (Table 1). Although not a specific goal of this project, we observed that the actual amount of specific allergen in each lyophilized suspension varied somewhat for each Der *f* 1 and Der *p* 1 suspension.

DISCUSSION

A new method has been developed to assess the effectiveness of fabrics to retain the dust mite allergens, Der *f* 1 and Der *p* 1. Both the fabrics were initially manufactured by the same process and each is composed of 100% cotton with a 300 thread count. One fabric remained untreated while the

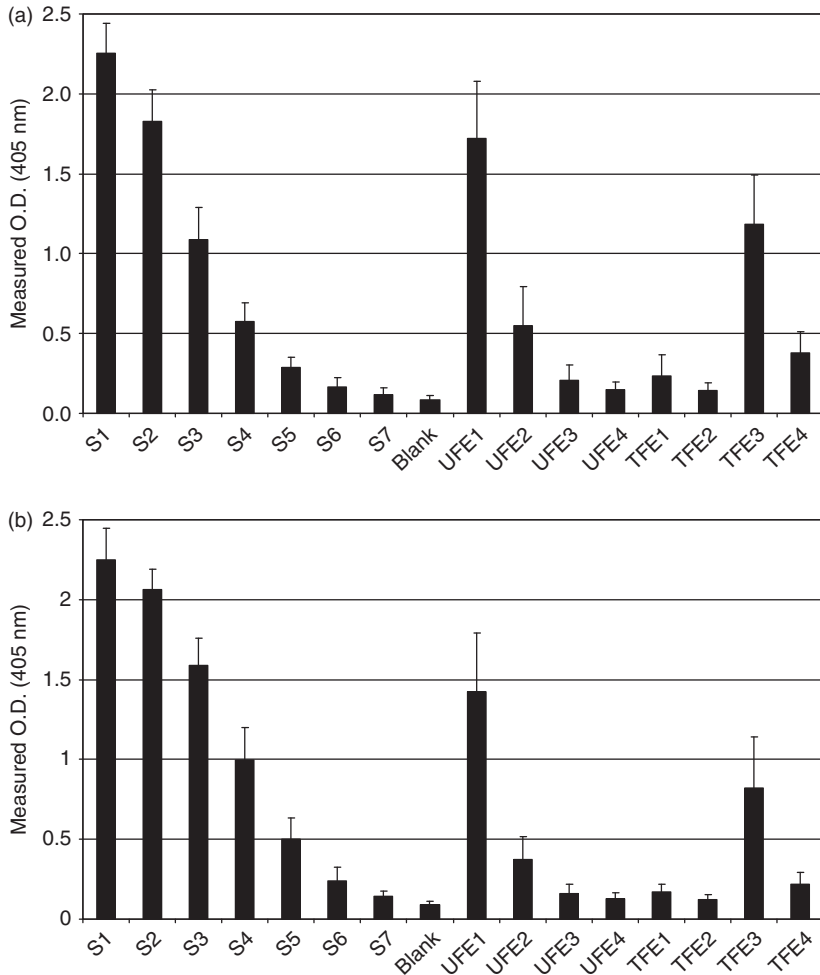


FIGURE 1. Assessment of *Der f* 1: (a) and *Der p* 1, (b) retention on treated and untreated fabrics using the new ELISA. S1–S7 are standard controls in duplicate. Blank is 10 μ L sterile 18-M Ω dH₂O with no antigen. UF is untreated fabric and TF is anionic fabric. E1–E4 are 10 μ L of extracts 1–4 as described in text. Standard error of the mean for the test materials is based on 25 samples.

other was treated to achieve an anionic state. Each sheet was cut in similar manner and sized similarly. All experiments were run concurrently and tested by a commercially available and accepted ELISA kit. Data derived from the ELISA was graphed to indicate the amount of detectable allergen

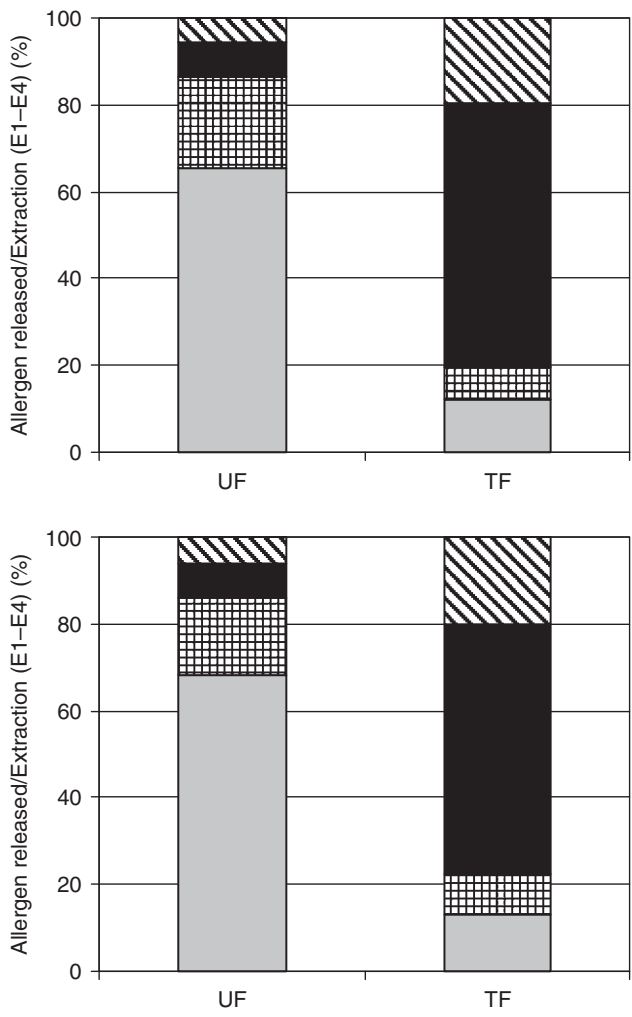


FIGURE 2. Percent release of *Der f* 1: (a) and *Der p* 1, (b) allergen from untreated fabric (UF) and treated fabric (TF) after four sequential extractions (E1, E2, E3, E4). The stacked graphs represent 100% of the allergen that was applied to the fabrics and indicate the percent release of total allergen from the treated and untreated fabrics after each of the four extractions.

in each ELISA test. Large bars represent greater allergen in the reaction than small bars.

Using our new approach, we demonstrated significantly different retention of dust mite allergens, *Der f* 1 and *Der p* 1, between the anionic treated and untreated fabric. Our graphed data clearly indicate a pattern

Table 1. Amount of specific allergen per extraction as measured by ELISA.

Extractions	Der <i>f</i> 1 (ng)		Der <i>p</i> 1 (ng)	
	U	T	U	T
E1	116 ± 0.36	12 ± 0.13	54 ± 0.37	5 ± 0.05
E2	30 ± 0.24	6 ± 0.05	12 ± 0.14	2 ± 0.03
E3	11 ± 0.09	70 ± 0.31	5 ± 0.06	26 ± 0.32
E4	6 ± 0.05	21 ± 0.13	3 ± 0.04	7 ± 0.08

of retention for both allergens by treated fabric that was significantly different than the pattern observed for untreated fabric. The graphs illustrate that the majority of extractable Der *f* 1 and Der *p* 1 allergen suspended in sterile dH₂O and dried onto untreated fabric will readily extract readily from after a single water extraction. Conversely, for the treated fabric, relatively small amounts of dried Der *f* 1 and Der *p* 1 were removed after two water extractions. Furthermore, significant removal of allergen from the treated fabric was achieved only after extractions with surfactant. In all cases, by the fourth extraction little allergen was extracted from either fabric.

Under most circumstances, controlling passage of dust mites is not an intended function of fabrics although barrier type bedding has been designed and evaluated [10,17,18,24,25]. However, the passage of dust mites is not the causative agent of allergy. Allergy or rhinitis is caused by particulates in the sheddings of dust mites that liberate allergens such as Der *f* 1 and Der *p* 1. Some of the barrier materials may inhibit the passage of larger fragments, but allergen is significantly smaller than 2–10 µm and their passage would not be inhibited by all of these bedding materials [17]. Trapping the allergen on the fabric itself would eliminate it from the breathing zone and might reduce incidence of allergy.

The possible uses of anionic surfaces to attract and retain biological molecules pose many questions on their effects and application. Anionic surfaces can be used for binding phospholipids components of biological membranes and may even act as a microbiocide [19–22,23]. However, we believe this is the first time this technology has been applied to bedding materials. The results from this new test suggests that anionic treatment of bedding materials are effective in controlling these two medically important allergens by retaining them on the fabric until it can be safely removed by laundering. Further testing is needed to evaluate the retention of other allergens and other anionic treated fabrics and materials.

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BIOGRAPHY



Dr Jonathan Black, a Research Microbiologist at RTI International (RTI), led the Microbial and Molecular Biology Department in the development of molecular- and tissue culture-based methods for the identification of important biodefense microbial pathogens using real-time quantitative polymerase chain reaction, molecular toxicity assays for mycotoxin characterization and potency, and cell lines for biomarker-based assays, and mitogenic challenge. In addition, he evaluates and implements novel molecular biology technologies for appli-

cations in sampling, isolation, quantitation, identification, and inactivation of microorganisms (bacteria, fungi, viruses) in human samples, air, water, soils, industrial fluids, and materials. He also investigates commercial and government-sponsored projects evaluating the efficacy of antimicrobials and biocides. Dr Black received his PhD in Microbiology in 1998 from North Carolina State University. Prior to joining RTI, he was a Postdoctoral fellow at both Duke University School of Medical School and at The University of North Carolina (UNC-CH), where he studied gene expression in mammalian cells in pursuit of prostate cancer and gene therapy treatments. Dr. Black passed away suddenly of pancreatic cancer in March 2009. His colleagues miss his keen with and sharp mind.