

***In vitro* evaluation of carboxymethylcellulose effectiveness in releasing *Candida albicans* from skin strips**

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Accepted for publication March 10, 2014.

Synopsis

Development of more effective products for cleansing the skin of microorganisms is enhanced by improved understanding of the mechanisms of attachment, as well as potential removal strategies. This paper describes an *in vitro* method for use of the cationic exchanger carboxymethylcellulose (CMC) to enhance the removal of the yeast *Candida albicans* as measured *in vitro* using human skin tape strippings. Several negatively charged and cationic exchanger compounds were evaluated using a visual release method to determine their ability to prompt release of *C. albicans* from human skin strips. CMC was further tested using a viable count method to assess its ability to effectively release the yeast from skin strips and to evaluate the effects of pH on CMC performance. CMC microparticles were found to release greater than 95% of bound *C. albicans* from the skin strips over a broad range of pH values. Interaction of CMC with the overall skin charge appears to result in detachment of *C. albicans*, a response suggesting that anionic particles may facilitate *C. albicans* removal from skin.

INTRODUCTION

Microorganisms attach to the skin through multiple mechanisms (1–4) and are frequently removed through complex physical cleaning strategies that combine surfactant action, shear forces, and affinity binding. Understanding mechanisms of microbial attachment to skin facilitates the design of physical cleaning products and compounds that counteract attachment forces, thereby freeing soils and microbes from the skin surface. In addition to the bacterial pathogens commonly encountered through fecal contact, approximately 30% of the general population, primarily women, suffer from health conditions caused by yeast and fungi (5–7).

The fungi represent a diverse, heterogeneous group of eukaryotes. Of the estimated one million species of fungi, molds, and yeasts, only about 50 are considered pathogenic (6,8). Most are opportunistic pathogens, becoming more prevalent during periods of stress or lowered immune response. Yeasts from the genus *Candida* are spread through contact with feces and secretions from the mouth, nose, skin, and vagina. Although normally

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noninfective and present in small numbers, *Candida* can multiply under some circumstances and cause symptoms requiring medical treatment. Circumstances that promote *Candida* infection include hormonal changes (5,9,10), medication side effects (5,11), and medical conditions (5,7).

The human body inhibits fungal growth through intact skin and other protective mechanisms, such as naturally occurring long-chain unsaturated fatty acids, pH, competition with normal bacterial flora, epithelial turnover, and the desiccated nature of the stratum corneum (11). Other body systems such as the respiratory tree, gastrointestinal tract, and vaginal vault are lined with mucous membranes (epithelium) bathed in antimicrobial fluids, and some are also lined with ciliated cells that actively remove foreign materials (8,12). Only when these protective barriers are breached can fungi gain access to, colonize, and multiply within host tissues. Candidiasis is a primary or secondary mycotic infection caused by *Candida*, often by the most common *Candida* species, *Candida albicans* (9). *Candida* is a dimorphic organism with the ability to convert between the yeast and mycelial fungal forms (10,13). The yeast form is a noninvasive sugar-fermenting organism. The mycelial fungal form produces long root-like invasive hyphae that penetrate the mucosa or skin (14).

Vaginitis, thrush (oral candidiasis), esophagitis, gastrointestinal candidiasis, cutaneous candidiasis, diaper rash, paronychia (nail fold infection), and chronic mucocutaneous candidiasis are all conditions caused by *Candida* (5,7,8). The clinical manifestations may be acute, subacute, chronic, or episodic. The etiology can be very difficult to determine because *Candida* spp. are commonly recovered from healthy people (5–7,9).

Candida has also been implicated as a frequent cause of infant diaper rash (5,7,8). Diaper rash usually begins as a solid patch of red, thickened skin, often around the anus, that can spread to cover the entire groin and lower buttocks. The same moist, warm, closed-off conditions triggering the original rash can also result in skin infection by the yeast. An infected rash can appear as a group of distinct round spots characterized by oozing, crusting, or surrounding redness and swelling.

Treatment of candidiasis almost always requires the use of antibiotics (11), which can result in the production of antibiotic resistant yeast, extended discomfort until the treatment achieves maximum effectiveness, and possibly harmful side effects due to pharmaceutical toxicity or allergies (11). Therefore, the development of effective physical methods of preventing or treating yeast infections without the use of antibiotics or antimicrobials is highly desirable. Technologies that interfere with the attachment to and invasion of the skin by yeast offer a promising strategy for physical control of yeast activity and prevention of related skin conditions.

Candida attaches to skin by three primary mechanisms: hydrophobic interaction (15), electrostatic interaction (4), and ligand interaction (4,12). These reactions may occur independently or in combination at any given moment during substrate interaction (1,15–17). Innovative strategies for disruption of yeast attachment mechanisms, which require a comprehensive understanding of yeast–skin interactions, are essential to the development of improved personal care products.

Hydrophobic interactions are weak chemical bonds formed through repulsion of insolvent molecules by water (18–20), mainly resulting from convergence of nonpolar side chains away from water (18). Hydrophobic interactions promote adherence of *Candida* to skin (12,15,21) and despite the weak nature of these bonds, their cumulative effect can

contribute substantially to the attachment of soil or yeast to the skin. Electrostatic interactions occur between charge density and distribution on a soil's surface and inversely charged components on another surface (17,19). Examples of electrostatic interactions include dipole–dipole forces, hydrogen bonds, cationic, and anionic interactions (4,16,22). Molecules with dipole moments attract each other electrostatically by aligning their positive and negative ends in close proximity (23). Hydrogen bonding occurs when a hydrogen atom is covalently bonded to an electronegative atom and also attracts an additional electronegative atom (5). The attraction created by individual dipole moments and hydrogen bonding should produce only small changes; however, the cumulative effects of simultaneous events may generate significant changes.

The biochemistry and structure of biological membranes cause them to be negatively charged (18); therefore, a cationic exchanger should repel yeast and an anionic exchanger should attract yeast, promoting removal from skin. Charge interactions are important to skin cleansing because alteration of the charge affinity between the soil and cleaning web or solution can increase cleaning effectiveness. This paper describes a method for use of the cationic exchanger carboxymethylcellulose (CMC) to promote enhanced removal of *C. albicans* from human skin.

MATERIALS AND METHODS

YEAST CULTURE

C. albicans (ATCC 10231) was subcultured using Sabouraud Dextrose (SAB-DEX) medium (Becton Dickinson, Cockeysville, MD) overnight at 37°C. The following day, 20 ml SAB-DEX broth was inoculated with 2–3 isolated *C. albicans* colonies and incubated at 32°C for 18 h with shaking at 220 rpm. The broth culture was diluted to 1×10^5 colony-forming units (CFU)/ml with 50 mM sterile potassium phosphate buffer pH 7.2 (VWR Industries, Batavia, IL).

VISUAL RELEASE PROTOCOL

The following protocol, outlined in Figure 1, was used to measure the effectiveness of various test materials (described in Table I) to induce release of *C. albicans* adhered to skin tape. Skin tape strips were produced by pulling D-Squame skin-sampling discs (CuDerm Corporation, Dallas, TX) four times from adjacent adult male volar forearm sites. The skin tape strips were placed into deep six-well plates (Becton Dickinson, Franklin Lakes, NJ), and the exposed adhesive was blocked with 2.0 ml of 5% bovine serum albumin (BSA; Sigma, St. Louis, MO) in phosphate buffered saline (PBS; 150 mM NaCl, 50 mM potassium phosphate, pH 7.4) for 60 min at 33°C while shaking at 220 rpm.

The fluid was removed from each well, the wells washed three times with 50 mM potassium phosphate, and 1.0 ml (10^5 CFU/ml) of *C. albicans* solution, prepared as described in the previous section, was added to each tape strip. Next, 1.0 ml of trypticase soy broth (TSB; Difco Labs, Detroit, MI) was added to each tape strip and the plates were incubated at 33°C for 60 min. The fluid was aspirated and the tape strips were washed three times with 3.0 ml tris(hydroxymethyl)aminomethane (TRIS)-buffered saline (TBS; 50 mM

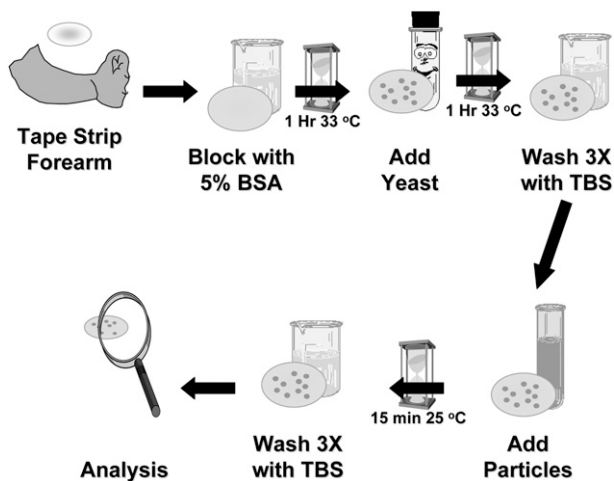


Figure 1. Diagram of yeast attachment assay.

Tris base, 150 mM NaCl, pH 7.4). Both sides of each tape strip were rinsed with a stream of TBS and placed in fresh 6-well plates. Test materials were suspended in TBS and 2.0 ml was added to the designated tape strip. The tape strips were incubated for 15, 30, or 60 min at 25°C while shaking at 220 rpm. The tape strips were then rinsed three times with 3.0 ml deionized (DI) water. Each tape strip was fixed by soaking in 2.0 ml of 2.5% glutaraldehyde (Sigma) for 10 min. The tape strips were then rinsed three times

Table I
Materials Used in Experiments

Material	Use concentration (mg/ml)	Exchange capacity	Size	Supplier
Benonite	8.0	NA ^a	Powder	Sigma, St. Louis, MO
Carboxymethyl (CM)-Cellulose	8.0	0.6 meq/g	Fibrous	Sigma, St. Louis, MO
Cellulose	8.0	NA	Fibrous	Sigma, St. Louis, MO
Diethylaminoethyl (DEAE)-Cellulose	8.0	1 meq/g	Powder	Sigma, St. Louis, MO
Diethyl-(2-hydroxypropyl) aminoethyl (QAE)-Cellulose	8.0	0.92 meq/g	Fibrous	Sigma, St. Louis, MO
Cellulose-Phosphate	8.0	3.8 meq/g	Powder	Sigma, St. Louis, MO
Chitin	8.0	NA	Powder	Sigma, St. Louis, MO
Carboxymethyl (CM)-Cellulose	0.4, 0.25	50–70 mg Lysozyme/g	3–4 µm	SCIGEN, Kent, UK
Polyethylenimine (PEI)-Cellulose	0.4	70–100 mg Albumin/g	3–4 µm	SCIGEN, Kent, UK
Diethylaminoethyl (DEAE)-Cellulose	0.4	70–100 mg Albumin/g	3–4 µm	SCIGEN, Kent, UK

^aNA = Not Applicable.

with 3.0 ml of DI water, and stained with 0.5 ml calcofluor white (Difco, Ann Arbor, MI) for 10 to 15 min. The tape strips were again rinsed three times with 3.0 ml DI water and allowed to air dry.

Once the tape strips were air-dried, the yeast cells were enumerated visually with an Olympus BH2 fluorescent microscope (Olympus Corporation, Lake Success, NY) fitted with a 405 nm excitation filter and a 455 nm barrier filter. The tape strips were placed with the white crescent label near the bottom edge of a microscope slide, perpendicular to the microscope objective. A 20X objective was used and the slide was positioned so that the field of view bisected the tape strip. Only the cells appearing within the field of view (an area of approximately $2 \times 10^7 \mu\text{m}^2$) were counted. The field of view represented 5% of the total tape strip area.

The percent removal was calculated as follows:

$$\frac{([\text{number of yeast on control tape strip}] - [\text{number of yeast on treated tape strip}])}{(\text{number of yeast on control tape strip})} \times 100$$

This method estimated approximately 10^4 yeast cells bound to a 22-mm diameter D-Squame tape strip under the described conditions. Tape strips were evaluated in triplicate for each condition. Digital photomicrographs were taken with a SPOT Digital Camera (Diagnostic Instruments, Sterling Heights, MI).

VIABLE COUNT RELEASE PROTOCOL

Skin tape strips produced using the previously described procedure were used to assess the ability of 3–4 μm CMC particles (described in Table I) to release *C. albicans* from the skin strips. The exposed adhesive on the tape strips was blocked with 2.0 ml of 5% bovine serum albumin (BSA) in PBS and incubated for 60 min at 33°C while shaking at 220 rpm. After the blocking solution was removed, each well was washed three times with potassium phosphate buffer (KP), and then 1.0 ml of *C. albicans* suspension (10^5 CFU/ml) and 1.0 ml of TSB were added to each tape strip. The plates were incubated at 33°C for 60 min, the fluid was aspirated, and the tape strips were rinsed three times with 3.0 ml TBS.

A suspension of 3–4 μm CMC (2.0 ml; 0.25 mg/ml in TBS) was added to each tape strip and 2.0 ml TBS was added to the control. The tape strips were incubated at 25°C while shaking at 220 rpm. At 0, 15, 30, and 60 min after addition of the CMC suspension, fluid samples were collected from each well (in triplicate) and plated on SAB-DEX agar. The agar plates were then incubated for 24 h at 33°C and the resulting yeast colonies were counted to determine the numbers viable of yeast in each solution.

EFFECT OF PH ON RELEASE EFFICACY

To determine the potential effect of pH on the release *C. albicans* from the skin by bath tissue containing CMC particles, skin tape strips were prepared as described previously. The exposed adhesive on the tape strips was then blocked with 2.0 ml of 5% BSA in PBS for 60 min

at 33°C while shaking at 220 rpm to cover exposed adhesive. Fluid was removed from each well and washed three times with KP, and then 1.0 ml of *C. albicans* suspension (10^5 CFU/ml) and 1.0 ml of TSB were added to each tape strip. The plates were incubated at 33°C for 60 min, the fluid was aspirated, and the tape strips were rinsed three times with 3.0 ml TBS.

CMC (3–4 μ m) was suspended (0.25 mg/ml) in each of the following buffers: citrate phosphate (0.1 M; pH 4.2, 5.2, and 6.2); TRIS (0.1 M; pH 7.2 and 8.2); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (50 mM; pH 6.2, 7.2, and 8.2); or phosphate (50 mM; pH 6.2, 7.2 and 8.2). A 2.0 ml sample of each CMC solution was added to one well containing a prepared tape strip, and 2.0 ml of each buffer without CMC were used as controls. The tape strips were then incubated at 25°C while shaking at 220 rpm. At 10-min time intervals after addition of the CMC or buffer control solutions, selected tape strips were rinsed three times with 3.0 ml DI water and fixed by incubating 2.0 ml of 2.5% glutaraldehyde with each sample for 10 min. The samples were then rinsed three times with 3.0 ml of DI water and stained using 0.5 ml calcofluor white for 10–15 min. The tape strips were again rinsed three times with 3.0 ml DI water and allowed to air dry. Once the tape strips were dry, the yeast cells were counted microscopically and the percent removal calculated using the method and formula described previously.

DETERMINATION OF *C. ALBICANS* GROWTH INHIBITION BY CMC

C. albicans (2.0 ml; 2×10^5 CFU/ml) in PBS was incubated with either 2.0 ml of 3–4 μ m CMC suspension (0.5 mg/ml) or 3–4 μ m cellulose–phosphate suspension in TBS (0.5 mg/ml; pH 7.2) for 210 min at 33°C. Fluid samples (1 ml) were collected at 0, 15, 30, and 60 min time intervals and plated (in triplicate) on SAB-DEX agar. The agar plates were incubated for 24 h at 33°C and the resulting colonies were counted.

PRETREATMENT OF SKIN TAPE STRIPS WITH CMC

Tape strips were prepared and the exposed adhesive was blocked with 5% BSA as previously described. Each tape strip was treated with 2.0 ml 3–4 μ m CMC suspension (0.25 mg/ml) in TBS for 15 min at 25°C at 220 rpm and then rinsed three times with 3.0 ml TBS. Next, 1.0 ml each of *C. albicans* suspension (10^5 CFU/ml) and TSB were added to each tape strip and incubated at 33°C for 60 min. The fluid was aspirated and the tape strips were rinsed three times with 3.0 ml of TBS. Each tape strip was then fixed with 2.0 ml of 2.5% glutaraldehyde for 10 min at 25°C while shaking at 220 rpm. The tape strips were then rinsed three times with 3.0 ml of DI water and stained with 0.5 ml calcofluor white for 10 to 15 min. The tape strips were again rinsed three times with 3.0 ml DI water and allowed to air dry. Once the tape strips were dry, the attached yeast cells were counted microscopically.

SIMULTANEOUS ADDITION OF YEAST AND CMC TO SKIN TAPE STRIPS

Tape strips were prepared and blocked with 5% BSA as previously described. Each tape strip was then treated with 1.0 ml each of 3–4 μ m CMC (0.5 mg/ml) and *C. albicans*

(10^5 CFU/ml) suspensions in TBS. The control was treated with *C. albicans* and TBS without 3–4 μm CMC. The tape strips were incubated for 1 h at 33°C while shaking at 220 rpm, and then rinsed three times with 3 ml of DI water. Each tape strip was fixed with 2.0 ml of 2.5% glutaraldehyde for 10 min. The tape strips were washed three times with 3.0 ml DI water and stained with 0.5 ml calcofluor white for 10–15 min. The tape strips were again rinsed three times with 3.0 ml DI water and allowed to air dry. Once the tape strips were dry, the yeast cells were counted microscopically.

STATISTICAL ANALYSIS OF RESULTS

The yeast counts recorded using the visual release protocol were subjected to further analysis. The average and standard deviation were calculated for the triplicate counts, and *t*-tests were conducted to determine the statistical significance of differences between the results for the different materials tested.

RESULTS

REMOVAL OF *C. ALBICANS* FROM SKIN TAPE STRIPS USING NEGATIVELY CHARGED PARTICLES

Experimental results (Tables II and III, Figures 2 and 3) indicated that *C. albicans* was removed from skin tape strips most effectively with 3–4 μm CMC particles. PEI-Cellulose and Benonite were only marginally effective, and the other materials evaluated did not release the yeast from the skin tape strips (Table III). As demonstrated by the results in Figure 2, yeast cells were rapidly released from the skin tape strips, within the first 10 min of contact with the CMC particles, as confirmed by the visual count observations (Table II). The numbers in the table reflect the average and standard deviation based on examination of three tape strips, visualizing $2 \times 10^7 \mu\text{m}^2$ or 5% of the tape strip surface area. The ability of CMC to effectively release yeast from skin tape strips is further supported by the photomicrographs shown in Figure 4.

EFFECT OF PH ON CMC EFFICACY

Anionic particles were able to displace the yeast from the skin tape strips over a wide range of pH (Figure 5). Although phosphate and HEPES buffers demonstrated reduced

Table II
Percent Removal of Yeast from Skin Tape Strips as Measured by Visual Observation (n = 3)

Material	Concentration (mg/ml)	Size (μm)	AVG	SD	Percent Removal
CM-Cellulose	0.4	3–4	4	2.8	97.7
PEI-Cellulose	0.4	3–4	109	12.0	36.6
DEAE-Cellulose	0.4	3–4	147	18.0	14.5

Table III
Percent Removal of Yeast from Skin Tape Strips as Measured by Visual Observation (n = 3)

Material	Concentration (mg/ml)	Size	AVG	SD	Percent Removal
Benonite	8.0	Powder	198.7	6.8	38.2
CM-Cellulose	8.0	Fibrous	317.0	24.8	1.5
Cellulose	8.0	Fibrous	294.7	21.2	8.4
DEAE-Cellulose	8.0	Powder	293.3	14.3	8.8
QAE-Cellulose	8.0	Fibrous	289.0	30.5	10.2
Cellulose-Phosphate	8.0	Powder	339.3	40.1	-5.5
Chitin	8.0	Powder	279.7	20.5	13.1

effectiveness at pH levels above 7.0, TRIS buffer remained highly effective at increased pH levels. The application of TRIS buffer could extend the efficacy of anionic particles to higher pH values.

ASSESSMENT OF CMC ANTIMICROBIAL EFFICACY

Neither charged particle significantly affected *C. albicans* viability (Table IV). Removal of yeast from skin tape strips using anionic particles as described does not kill or inhibit the growth of the microbes.

PRETREATMENT OF SKIN TAPE STRIPS WITH CMC

Pretreatment of the skin tape strips with CMC did not prevent yeast attachment, as established by the numbers of yeast cells counted on treated and untreated tape strips (Table V). A *t*-test on the average numbers of yeast cells observed for the triplicate treated and untreated strips indicated no significant differences (*p* = 0.264).

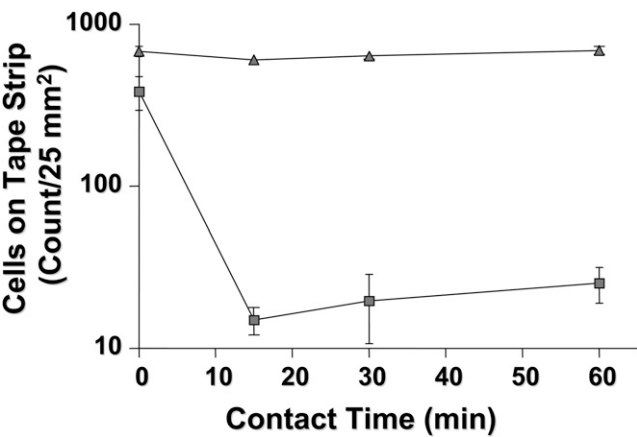


Figure 2. Visual counts of *C. albicans* on tape strips after exposure to 3–4 μ m CMC particles; ■ = Skin treated with 0.25 mg/mL CMC; ▲ = Untreated skin; Bars = SD; (n=3).

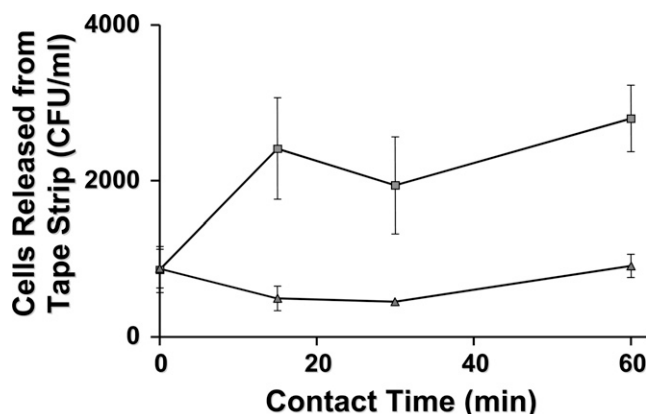


Figure 3. *C. albicans* release from tape strips as a function of time. Samples were exposed to 3–4 μm CMC particles, and percent release was determined using the viable count method to compare treated and untreated samples. ■ = Skin treated with 0.25 mg/mL CMC; ▲ = Untreated skin; Bars = SD; (n=3).

SIMULTANEOUS ADDITION OF YEAST AND CMC TO SKIN TAPE STRIPS

When added during the process of yeast attachment, CMC inhibited the attachment process (Table VI). This result indicates a possible application of charged particles as a preventative measure to inhibit yeast attachment to the skin tape strips.

DISCUSSION

Bacteria, fungi, and soil particles adhere to the skin despite their negative charges, which should repel the negatively charged skin (3,4,15,18). Many different binding mechanisms contribute to the binding of microbes to the skin surface, and the combined strength of these adhesion interactions is far greater than that of any single attachment mechanism (3,12,20). Surfactants and solvents are effective and commonly used methods of cell release from surfaces; however, they can be harsh and potentially damaging to human skin. Even minimal use of detergents and organic solvents can

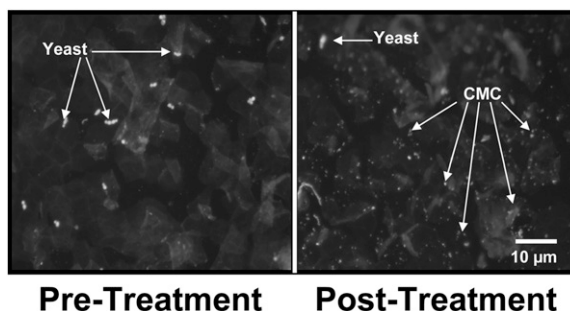


Figure 4. *C. albicans* cells (white rods) are displaced by CMC particles. Photomicrographs of skin before and after treatment with 3–4 μm CMC particles (0.25 mg/mL) for 20 min at 25°C were taken with Olympus BH2, UV excitation, and a SPOT digital camera.

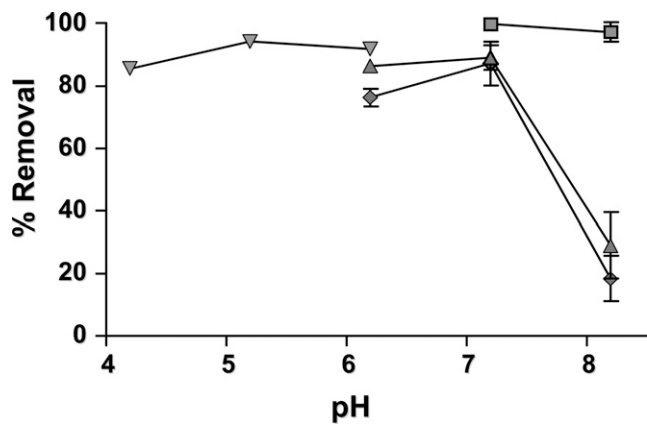


Figure 5. Effects of pH on removal of yeast from tape strips using 3–4 μ m CMC particles (0.25 mg/ml) as determined by visual counts; ▼ = Citrate-Phosphate Buffer; ◆ = Phosphate Buffer; ▲ = HEPES Buffer; ■ = TRIS Buffer; Bars = SD (n=3).

greatly impact the integrity of the stratum corneum barrier and potentially cause skin irritation (24).

Electrostatic removal of contaminants from the skin surface, using negatively charged particles to reduce the attractive forces between the contaminant and the skin, offers a potential method to clean the skin with reduced risk of skin irritation. Negatively charged CMC particles (3–4 μ m), as well as other anionic materials or cationic exchangers, in close proximity to the attached microbe appear to act electrostatically on the skin tape strips and attached *C. albicans* with sufficient strength to overcome the combined binding affinity between them (Figures 1 and 3). The released microbe can then be transferred to the cleaning substrate (wipe, diaper, cloth) to facilitate contaminant removal. The CMC microparticles do not exhibit biocidal or biostatic properties (Table IV) and are not anticipated to have harsh or damaging effects on the skin.

C. albicans, a causative agent of diaper rash known to have multiple mechanisms (electrostatic, hydrophobic, and adhesin interactions) of attachment to mammalian skin (4,12,15), was removed from skin cells bound to a tape strip by what appears to be an electrostatic interaction (Figures 1 and 3). After a 10-min exposure to the CMC particles, greater than 95% of the bound yeast was removed from the skin tape strips (Table II) based on visual

Table IV
Viability of *C. albicans* When Exposed to CMC or Cellulose Phosphate^a

Exposure time (min)	CFU/ml		
	Control	CMC	Cellulose phosphate
0	1.12 × 10 ⁵	1.07 × 10 ⁵	1.12 × 10 ⁵
15	1.29 × 10 ⁵	1.27 × 10 ⁵	1.35 × 10 ⁵
30	1.31 × 10 ⁵	1.14 × 10 ⁵	1.18 × 10 ⁵
60	1.27 × 10 ⁵	1.29 × 10 ⁵	1.66 × 10 ⁵
210	8.45 × 10 ⁴	9.97 × 10 ⁴	1.35 × 10 ⁵

^a210-min exposure; n = 3.

Table V
Visual Cell Counts of *C. albicans* on CMC Treated and Untreated Tape Strips^a

CMC treated skin tape strips	Untreated skin tape strips
840 ± 55	904 ± 25

^aAverage ± SD, n = 3, count per 2x10⁷ μm²; No significant difference between treated and untreated $p = 0.264$ (*t*-test).

examination of triplicate tape strips. The CMC particles are believed to interact with the overall net negative charge of the skin cells, overcoming the combined adhesion forces and causing detachment of microorganisms adhered to the skin stripping. The removal of yeast from skin tape strips using CMC is highly effective and offers greater removal efficiency as compared to larger or alternative charged particles (Tables II and III). Anionic particles, in this model, were demonstrated to facilitate the release of yeast attached to skin tape strips (Tables II and III; Figures 1–3) over a wide range of pH.

Although the results of the *t*-test (CMC vs. PEI-Cellulose, $p = 6.0 \times 10^{-5}$; CMC vs. DEAE-Cellulose, $p = 8.0 \times 10^{-5}$; DEAE-Cellulose vs. PEI-Cellulose, $p = 0.02$) indicate that a larger sample size would be more informative, the CMC cellulose appears to perform better than the other ion exchange materials. The difference observed between the DEAE-Cellulose and PEI-Cellulose would likely be eliminated with a larger sample size. The difference between the CMC and the other materials is very large and is anticipated to remain significant even with a larger sample size. In combination with the visual observation confirming the loss of yeast from the skin tape strips treated with CMC, these results suggest that CMC shows greater potential for release of yeast cells than other ion exchange types under the conditions of this study.

This process offers potential advantages over traditional cleaning compositions, in part, because the contaminant would be not merely dislodged from the skin surface, but would be first dislodged and then removed from the skin's surface through subsequent binding with the cleansing material. Therefore, interaction between contaminants and charge-altered cleaning compositions may involve an actual energy transfer as energy is released and recaptured in the dislodging and rebinding of contaminants from the skin surface to the cleaning product.

CONCLUSIONS

Delivery of negatively charged CMC particles to skin tape strips under the described conditions facilitates release of attached microorganisms and/or soil rapidly and over a

Table VI
Reduction of *C. albicans* Attachment to Skin Tape Strips When Yeast and CMC Were Added Simultaneously^a

CMC + Yeast	Yeast
71	429
72	568
147	498

^aCount per 2 × 10⁷ μm²; 30-min exposure.

wide pH range; however, additional studies are needed to determine the effectiveness of CMC for removal of other types of microbes, with shorter contact times, or after longer periods of microbial attachment. Other potential effects of CMC on human skin, such as irritation or effects resulting from removal of beneficial microbes, would also need to be explored. Through incorporation of anionic particles, existing cleansing compositions could potentially be altered to improve their performance. In addition, microbial removal technologies based on electronic charge, as opposed to current chemical and physical removal methods, could result in development of new products with reduced potential for irritation. Anionic particles could be incorporated into a variety of delivery matrices such as solutions, lotions, or solid substrates including woven web, non-woven web, spun-bonded fabric, melt-blown fabric, knit fabric, wet-laid fabric, needle-punched web, cellulosic material, or any combination thereof. Potential applications of CMC cleaning technology include wet wipes, bath tissue, facial tissue, infant diapers, adult incontinence products, lotions, liquid skin cleaners, and other commercially available skin cleaning products. This technology could also be useful for hard surface cleaning, home health care applications, industrial cleaning, and veterinary applications.

ACKNOWLEDGMENTS

The author thanks Laura Mallary for her helpful comments on the manuscript. The author is employed by Kimberly-Clark Corporation. Support for the studies described in this paper was provided by Kimberly-Clark Corporation.

REFERENCES

- (1) L. D. Renner and D. B. Weibel, Physicochemical regulation of biofilm formation, *MRS Bull.*, **36**, 347–355 (2011).
- (2) H. J. Busscher, M. M. Cowan, and H. C. van der Mei, On the relative importance of specific and non-specific approaches to oral microbial adhesion, *FEMS Microbiol. Rev.*, **8**, 199–209 (1992).
- (3) P. Gilbert, D. J. Evans, E. Evans, I. G. Duguid, and M. R. M. Brown, Surface characteristics and adhesion of *Escherichia coli* and *Staphylococcus epidermidis*. *J. Appl. Bacteriol.*, **71**, 72–77 (1991).
- (4) G. Cotter and K. Kavanagh, Adherence mechanisms of *Candida albicans*. *Br. J. Biomed. Sci.*, **57**, 241–249 (2000).
- (5) V. Krcmery and A. J. Barnes, Non-albicans *Candida* spp. causing fungaemia: Pathogenicity and antifungal resistance, *J. Hosp. Infect.*, **50**, 243–260 (2002).
- (6) D. K. Morales and D. A. Hogan, *Candida albicans* interactions with bacteria in the context of human health and disease, *PLoS Pathog.*, **6**, e1000886 (2010).
- (7) A. Rashid and M. D. Richardson, "Pathogenesis of Dematophytosis," in *Cutaneous Infection and Therapy*, R. Aly, E. R. Beutner, and H. Maibach, Eds. (Marcel Dekker, New York, 1997) pp. 127–139.
- (8) D. E. Babel, "Dermophytes and Nondermatophytes: Their Role in Cutaneous Mycoses," in *Cutaneous Infection and Therapy*, R. Aly, E. R. Beutner, and H. Maibach, Eds. (Marcel Dekker, New York, 1997), pp. 191–198.
- (9) C. Westwater, D. A. Schofield, P. J. Nicholas, E. E. Paulling, and E. Balish, *Candida glabrata* and *Candida albicans*; dissimilar tissue tropism and infectivity in a gnotobiotic model of mucosal candidiasis, *FEMS Immunol. Med. Microbiol.*, **51**, 134–139 (2007).
- (10) D. S. Thompson, P. L. Carlisle, and D. Kadosh, Coevolution of morphology and virulence in *Candida* species, *Eukaryot. Cell*, **10**, 1173–1182 (2011).
- (11) D. Roberts, "The Risk/Benefit Ratio of Modern Antifungal Pharmacological Agents," in *Cutaneous Infection and Therapy*, R. Aly, E. R. Beutner, and H. Maibach, Eds. (Marcel Dekker, New York, 1997), pp. 183–190.

- (12) D. Poulain and T. Jouault, *Candida albicans* cell wall glycans, host receptors and responses: Elements for a decisive crosstalk, *Curr. Opin. Microbiol.*, **7**, 342–349 (2004).
- (13) I. D. Jacobsen, D. Wilson, B. Wächtler, S. Brunke, J. R. Naglik, and B. Hube, *Candida albicans* dimorphism as a therapeutic target, *Expert Rev. Anti Infect. Ther.*, **10**, 85–93 (2012).
- (14) E. Hiller, M. Zavrel, N. Hauser, K. Sohn, A. Burger-Kentscher, K. Lemuth, and S. Rupp, Adaptation, adhesion and invasion during interaction of *Candida albicans* with the host—focus on the function of cell wall proteins, *Int. J. Med. Microbiol.*, **301**, 384–389 (2011).
- (15) A. M. Gallardo-Moreno, M. L. González-Martín, C. Pérez-Giraldo, E. Garduño, J. M. Bruque, and A. C. Gómez-García, Thermodynamic analysis of growth temperature dependence in the adhesion of *Candida parapsilosis* to polystyrene, *Appl. Environ. Microbiol.*, **68**, 2610–2613 (2002).
- (16) C. I. Pereni, Q. Zhao, Y. Liu, and E. Abel, Surface free energy effect on bacterial retention, *Colloids Surf., B*, **48**, 143–147 (2006).
- (17) J. Ubbink and P. Schär-Zammaretti, Probing bacterial interactions: Integrated approaches combining atomic force microscopy, electron microscopy and biophysical techniques, *Micron*, **36**, 293–320 (2005).
- (18) J. B. Reece, L. A. Urry, M. L. Cain, S. A. Wasserman, P. V. Minorsky, and R. B. Jackson, *Campbell Biology*, 9th Ed. (Pearson Benjamin Cummings, San Francisco, 2010).
- (19) B. V. Derjaguin and L. Landau, Theory of the stability of strongly charged lyophobic soils on the adhesion of strongly charged particles in solutions of electrolytes, *Acta Physicochem.*, **14**, 633–662 (1941).
- (20) M. Cuéllar-Cruz, A. Vega-González, B. Mendoza-Novelo, E. López-Romero, E. Ruiz-Baca, M. A. Quintanar-Escorza, and J. C. Villagómez-Castro, The effect of biomaterials and antifungals on biofilm formation by *Candida* species: A review, *Eur. J. Clin. Microbiol. Infect. Dis.*, **31**, 2513–2527 (2012).
- (21) J. R. Naglik, S. J. Challacombe, and B. Hube, *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis, *Microbiol. Mol. Biol. Rev.*, **67**, 400–428 (2003).
- (22) M. Katsikogianni and Y. F. Missirlis, Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions, *Eur. Cell Mater.*, **8**, 37–57 (2004).
- (23) S. S. Zumdahl and S. A. Zumdahl, *Chemistry*, 8th Ed. (Brooks Cole, Belmont, 2008).
- (24) W. Yan-yu, W. Xue-min, T. Yi-Mei, C. Ying, and L. Na, The effect of damaged skin barrier induced by subclinical irritation on the sequential irritant contact dermatitis, *Cutan. Ocul. Toxicol.*, **30**, 263–271 (2011).

