EFFECT OF CHITOSANS ON SKIN-BORNE MICROORGANISMS

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INTRODUCTION

Chitosan is an abundant biopolymer, consisting of poly [β-(1→4)-2-amino-2-deoxy-D-glucopyranose] — which is obtained from the exoskeletons of crustaceans, arthropods and molluscs, as well as the cell walls of certain fungi [1]. Chitosan (and chitosan oligomers) have recently attracted considerable interest due to their biological activities (antimicrobial, antitumor and hypocholesterolemic functions), attributable to a unique polycationic nature [2]. Its production in nature has been estimated to be ca. 10^9-10^{10} ton/year [3]. Application in the textile industry, as an antimicrobial finish, became popular due to its ability to provide protection against allergies and infectious diseases, coupled with moisture retention and wound healing capabilities.

Healthy human skin has a normal resident flora, which is typically non-pathogenic. Bacterial genera isolated are normally coagulase-negative staphylococci and diphtheroid rods on the skin surface, and propionibacteria in the infundibulum of the sebaceous glands [4]. The distribution and density of said flora is dependent on age, and such environmental factors as sebum secretion, occlusion, temperature and humidity. However, when some skin disorders occur such as atopic dermatitis, colonisation of the skin by *Staphylococcus aureus* may trigger, or even intensify the condition. Atopic dermatitis is the most severe and chronic type of eczema affecting 10-20% of the world population. This disorder produces skin inflammation, and interferes with the skin's ability to retain adequate levels of moisture. The efficacy of chitosan as an antimicrobial agent in textiles has been confirmed by several studies [5, 6]; however limitations include the hand touch of fabrics, which is adversely affected when high molecular weight chitosans are used, coupled with the low laundering durability. Antimicrobials may reduce the density of the microflora resident on the skin, but they do not completely eliminate it. While antimicrobials may cause irritant and allergic contact dermatitis, no evidence exists that the use of antimicrobial substances may change the ecology of resident bacteria on the skin — thereby leading to overgrowth of pathogenic bacteria [4].

Textile materials are a rather suitable environment for bacterial growth — especially such natural fibers as cotton, due to their porous hydrophilic structure, which is able to retain water, oxygen and nutrients [7]. However, the aforementioned growth can induce serious consequences in situations where microbial growth/contamination needs a strict control. Schools and hospitals have, in particular, for long recognized the need to inhibit/reduce microbial growth when aiming at the comfort and health of people. Although antimicrobial products have been used for many years, only recently have they been extensively used in textiles. However, an effective antimicrobial for the textile industry (especially if applied to garmets) can not just kill or repel microorganisms; it must do so safely and selectively, hence not compromising the health of the person wearing it. At the same time, such an increased demand for antimicrobial products warrants deeper studies encompassing their activity upon selected microorganisms, before a clear assessment on their efficacy can be made.

In this work, the primary aim was to assess the antimicrobial effect of two different molecular weight chitosans, upon microorganisms actually isolated from the skin of healthy individuals, following impregnation onto cotton fabric.

MATERIALS and METHODS

Sampling

The skin area (5 x 5 cm^2) of the inner elbow of 24 persons without any known skin disorder, plus one (as positive control) diagnosed with atopic dermatitis, were duly sampled. These samples were plated on nutrient and mannitol salt agar (Merck, Germany). From each sample, 16 colonies were selected at random using the Harrison disk method [8], or all available colonies (if less than 16 were available), and purified for further identification — hence comprising a total of 600 isolates. From these, the virulence factors of 50 selected isolates (all catalase positive and oxidase negative) were studied; from these 13 (i.e. 10 from healthy individuals and 3 from the atopic one) were, in turn, used for evaluation with the cotton fabric. In addition, 2 reference strains were used as controls, *S. aureus* (ATCC 25923) and *S. epidermidis* (ATCC 155), thus comprising a total of 15 isolates in the study.

Fabric preparation

Two concentrations (0.1 and 1 % (w/v)) of chitosans, with two molecular weights — designated as low (ca. 150 kDa) and high (ca. 600 kDa), were impregnated into rounds (ca. 4.8 cm dia.) of organic cotton fabric (Crispim Abreu, Riba de Ave, Portugal). The chitosan solutions were previously prepared in 1 %(v/v) acetic acid, and allowed to dissolve for 24 h at 50^o C; the pH was then adjusted to 5.6-5.8 with NaOH (10 M). The solutions were autoclaved (at 121^o C, for 20 min), and the cotton rounds were impregnated following the pad-dry-cure method (Shin, 2000). The impregnated fabric rounds were finally sterilized by exposure to UV for 15 min.

Antimicrobial activity

The assessment of the antimicrobial activity was carried out according to the standard procedure described in the AATCC test method 100-2004 [9]. Rounds of cotton fabric, impregnated as described above, were accordingly inoculated with 1 mL of a bacterial suspension containing ca. 10^5 CFU/mL, and then incubated at 37^o C. Standard plate counts were performed by 1, 6 and 24 h of incubation, and the percent reduction (R) was determined as $R = 100\ (B\text{-}A)/B$ — where A represents the number of bacteria recovered from the inoculated treated rounds incubated over the desired contact period, and B represents the number of bacteria recovered from the inoculated treated rounds immediately after inoculation.

RESULTS and DISCUSSION

The distribution of the mannitol-positive and -negative staphylococci, isolated from the skin of normal individuals and one with atopic dermatitis, is shown in Fig 1. In healthy individuals, the incidence of mannitol-negative staphylococci (60.1%) overtakes that of mannitol positive species (39.9%); to a proportion similar to that reported by Aly et al. [10]. In the case of the atopic individual, our results coincide again with those of Aly et al., with the ratio of mannitol-positive to -negative staphylococci of 94.5/5.5%.

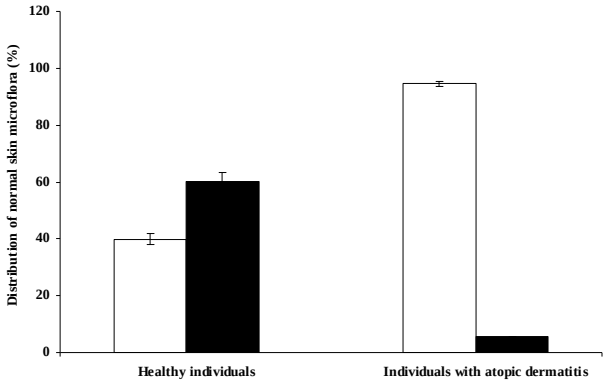

Figure 1. Percent distribution of staphylococci amongst healthy individuals, and that of the individual with atopic dermatitis: -□- mannitol-positive staphylococci, and -■- mannitol-negative staphylococci.

From the whole pool of isolates, 50 were selected (Table 1) based on their representativity (making sure that isolates obtained from every individual were present). From these, 13 were further selected (based on combination of virulence factors) to evaluate the efficacy of the chitosan impregnated fabrics. From inspection of Table 1, one concludes that 52% of the selected isolates were coagulase-positive, 30% were DNAse positive, 40% had protein A, 82% were β-hemolytic, 18% were γ-hemolytic, and 2% produced enterotoxin. Furthermore, the preliminary identification allowed one to conclude that the prevalent species of the human skin normal microflora comprises *S. aureus, S. epidermidis*, *S. cohnii*, *S. saprophyticus* and *S. lentus*. Isolates 48, 49 and 50 were isolated from the individual with the atopic condition, whereas the others originated from healthy individuals.

Table 1. Virulence factors (mannitol fermentation, coagulase, DNAse, Protein A, hemolysis and enterotoxin) in the selected isolates from the skin of tested individuals.

Isolate	Mannitol fermentation/coagulase	DNAse	Protein A	Hemolysis	Enterotoxin
1	-/-	-	-	b	-
2	+/+	+	+	b	+
3	-/-	+	-	b	-
4	+/+	-	-	b	-
5	+/-	-	-	b	-
6	-/-	+	-	b	-
7	-/-	-	-	b	-
8	+/+	-	-	b	-
9	-/-	-	-	b	-
10	+/-	-	+	b	-
11	+/-	-	-	g	-
12	+/-	+	+	b	-
13	+/-	-	+	b	-
14	+/-	-	-	g	-
15	-/-	+	+	b	-
16	-/-	+	-	b	-
17	+/+	-	+	g	-
18	+/+	-	+	b	-
19	+/+	-	+	b	-
20	-/-	-	-	b	-
21	+/+	-	-	b	-
22	+/+	-	-	b	-
23	+/+	-	-	b	-
24	-/-	-	-	g	-
25	-/-	-	-	g	-
26	-/-	-	-	b	-
27	+/-	-	-	b	-
28	+/+	+	+	b	-
29	+/+	+	-	b	-
30	+/+	-	-	g	-
31	+/+	+	+	b	-
32	+/+	+	+	b	-
33	+/-	-	-	g	-
34	+/-	-	-	b	-
35	+/+	-	-	g	-
36	+/+	-	-	b	-
37	-/-	-	-	b	-
38	+/+	-	-	b	-
39	+/+	+	+	g	-
40	+/+	-	+	b	-
41	+/+	-	+	b	-
42	-/-	-	-	b	-
43	+/+	-	-	b	-
44	+/+	-	+	b	-
45	-/-	-	+	b	-
46	-/-	-	-	b	-
47	+/+	+	+	b	-
48	+/+	+	+	b	-
49	+/+	+	+	b	-
50	+/+	+	+	b	-

The percent reduction of the bacterial numbers of the selected isolates, following contact with two chitosans (at two concentrations) impregnated onto cotton fabric rounds, is depicted in Table 2. The high molecular weight chitosan was the most effective — with bacterial viable numbers decreasing as chitosan concentration increases, as also determined by other research groups [5, 11]. Isolates originated either from healthy or atopic individuals showed distinct behaviours when acted upon by the low MW chitosan only: the latter seemed more efficient towards isolates from the atopic individual (with viable numbers decreasing in some instances, by ca. 6 log cycles) than from healthy ones (ca. 3 log cycles), hence suggesting a possible role upon control of pathogenic microorganisms in these individuals.

Table 2. Percent reduction in viable numbers of 15 isolates following 6 h of contact with low and high MW chitosans, at 0.1 and 1 % (w/v). Inhibition rates below 70% are represented in bold.

Microorganism	Mannitol fermentation/coagulase	Chitosan			
		High MW		Low MW	
		1%	0.1%	1%	0.1%
S. epidermidis *	-/-	95.58	**63.22**	98.73	98.31
S. aureus *	+/+	95.7	**70.2**	81	95.71
2	+/+	97	99.55	**29.39**	92.82
4	+/+	83.99	89.77	97.21	92.04
11	+/-	74.96	97.34	71.53	99.89
14	+/-	100	**55.96**	91.22	92.04
15	-/-	99.11	**15.93**	**35.23**	99.89
16	-/-	94.84	79.06	97.66	78.59
30	+/+	99.11	99.37	100	**52.25**
31	+/+	97.09	**44.12**	96.15	**63.79**
37	-/-	100	**53.28**	100	71.46
41	+/+	100	**12.06**	100	93.42
48	+/+	98.35	77.97	84.82	97.72
49	+/+	100	**67.74**	98.44	90.8
50	+/+	99.94	92.86	99.47	**52.94**

According to previous results [5], the anti-microbial activity was labelled as efficient when a percent reduction above 70% was observed. From table 2, it is apparent their bactericidal efficacy towards some of the tested isolates (i.e. 100% reduction). Surprisingly, this trend did not hold for the lower concentration tested (0.1 %); and cases existed for which the lower MW chitosan was actually more effective than the higher one.

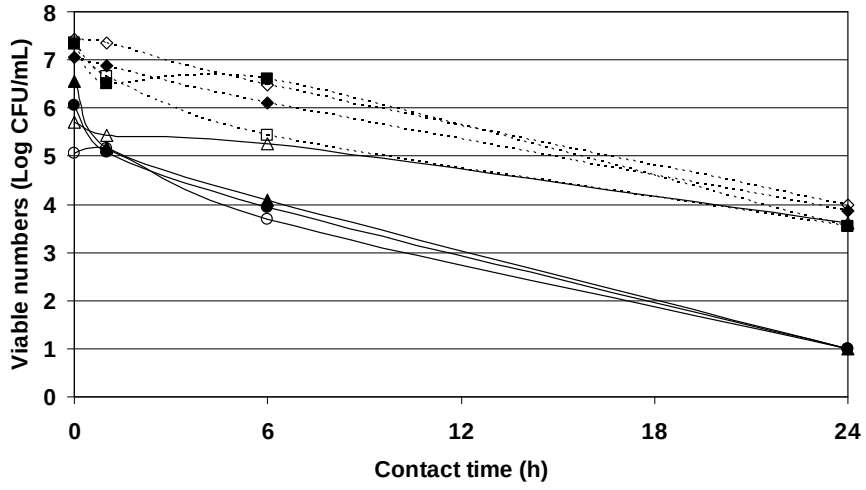

Figure 2. Decrease in viable numbers of control *Staphylococcus aureus* (broken lines) and control *Staphylococcus epidermidis* (filled lines), when in contact with high (open symbols) and low (closed symbols) molecular weight chitosans for a 24 h-period: –□- *Staphylococcus aureus*, 1%; -○- *Staphylococcus epidermidis*, 1%; -◇- *Staphylococcus aureus*, 0.1%; -△- *Staphylococcus epidermidis*, 0.1%.

From the above graph, it can be observed that the behaviour of two species represented are quite distinct — with *S. epidermidis* (coagulase-negative, and bearing fewer virulence factors) numbers decreasing ca. 4 log cycles within 24 h, whereas *S. aureus* decreased ca. 3 log cycles. Again, the existence of more virulence factors may be related to its greater resistance to anti-microbials. However, the anti-microbial effect of chitosan seemed to be independent of the existence of different virulence factors (see Tables 1 and 2).

The amount of polymer that adhered to the cotton fabric may be a determinant factor — since this could interfere with anti-microbial efficacy. The dry weight was determined before and after impregnation; the rounds with 1 % (high MW) solution absorbed 0.025 g on average, whereas those with 0.1 % absorbed 0.036 g; those with 1 % (low MW) absorbed 0.033 g whereas those with 0.1 % absorbed 0.028 g. Therefore, fabric rounds embedded with 1 % (high MW) absorbed less polymer, but were more efficient, unlike those embedded in 0.1 % (high MW) — that absorbed more and caused lower reduction rates. With the low MW solutions, the opposite occurred: rounds embedded in 1 % solution absorbed more than with 0.1 %, which in turn showed higher reduction rates than the 0.1 % ones. In the case of Gram-positive bacteria, while high MW chitosans are believed to form a barrier around the cell which impedes nutrient uptake (thus leading to cell death), lower MW chitosans were able to penetrate the cell, and hence disturbing its physiological activities and eventually killing them [6].

The use of biopolymers as chitosan, as anti-microbial agents in the textile industry thus appears promising toward control and prevention of skin diseases (such as atopic dermatitis) — as it can selectively and effectively kill skin-borne microorganisms. This selectivity can be an advantage, since the disruption of the natural microbial balance on the skin is a major concern pertaining to the use of antimicrobials.

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