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Sustained antibacterial effect of a hand rub gel incorporating chlorhexdine-loaded nanocapsules (Nanochlorex®)

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Abstract

In the present study, an original chlorhexidine-loaded nanocapsule-based gel (Nanochlorex®) was tested as hand rub gel against the resident skin flora in comparison with 2-propanol 60% (v/v) and 62% (v/v) ethanol-based gel (Purell®). After 30-s hand rub, the immediate bactericidal effect of Nanochlorex® was found comparable to 2-propanol 60% (v/v) (reduction factor, RF: 0.30 ± 0.35 versus 0.38 ± 0.55, $P > 0.05$) against aerobic bacteria, whereas the post-values of surviving anaerobes were shown significantly lower from Nanochlorex® ($P < 0.001$) and insignificant from 2-propanol 60% (v/v) ($P > 0.05$). Sustained antibacterial effect of Nanochlorex® was confirmed against the resident and transient hand flora in two sets of experiment. In the first, the results obtained with the glove-juice technique showed that the bactericidal effect induced by Nanochlorex® hand rub persisted throughout 3-h period, while Purell® failed to reduce significantly the post-values of surviving bacteria. In the second, repeated artificial contaminations with *Staphylococcus epidermidis* was carried out onto *ex vivo* human skin pre-treated by either Nanochlorex® or Purell® for 5 min, then maintained in cell diffusion apparatus for 4 h. The $\log_{10}$ reduction of surviving bacteria was significantly higher with Nanochlorex® than that determined with Purell® after three successive contaminations (from ~5.5 to 1.5 $\log_{10}$ reduction for Nanochlorex® between the first and the third contamination; ~1 $\log_{10}$ reduction for Purell® throughout the experiment), confirming the sustained antibacterial effect of chlorhexidine-loaded nanocapsule-based gel. The immediate and sustained antibacterial effect of Nanochlorex® was explained by chlorhexidine carrier system which improved the drug targeting to bacteria and reduced from osmotic gel further bacterial growth on the skin. Nanochlorex® might constitute a promising approach for hygienic hand disinfection in care practice performing multiple procedures.

Keywords: Hand rub; Chlorhexidine; Nanoparticles; Alcohol-based gel; *Staphylococcus epidermidis*

1. Introduction

The avoidance of nosocomial infections involves the improvement of hand hygiene practices and the reduction of the transmission of pathogenic microorganisms to patients and personnel, as reported by a new Centers for Disease Control and Prevention guideline for hand hygiene in health care settings (Boyce and Pittet, 2002). The transfer of pathogenic bacteria via the hands of staff in health care centers, known as a source of outbreaks of nosocomial infections (Maillard et al., 1998; Messager et al., 2001), might be significantly reduced by three procedures (Kampf and Kramer, 2004): (i) the *social hand wash* with plain soap and warm water, e.g., when hands are visibly contaminated with dirt, soil or organic material, before and after each contact with the patients, after moving from a contaminated to a clean area during care of an individual patient; (ii) the *hygienic* (Europe) or *antiseptic* (United States) *hand*

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wash using antimicrobial or medicated soap and water (then, so-called "scrub" formulations), e.g., before and after each contact with the patients in critical care units, immunocompromised, or with large wounds or burns, when hands are inadvertently contaminated with a heavy microbial load; (iii) the *hygienic hand disinfection* (Europe) ensuing the application of an alcohol (e.g. ethanol, 1-propanol, 2-propanol, 60–70%, v/v)-based hand rub product (solution or gel) into dry and visibly "clean" hands without water (then, so-called "rub" formulations). Therefore, the bactericidal efficacy of hygienic hand wash (rinse-off) and hygienic hand disinfection (leave-on) products are tested in Europe according to EN 1499 and EN 1500 standards (Rotter, 2004). Although hygienic hand wash and hygienic hand disinfection are accepted as the main methods allowing to break the chain of transmission (Kampf et al., 1998; Maillard et al., 1998; Messager et al., 2001), the frequent use of antimicrobial or medicated soap can be the cause of skin irritation or allergies (Messager et al., 2001).

Hence, new strategies encompassing numerous drawbacks related to misuse of biocides are subject to extensive studies. In this field, drug carrier systems (e.g. liposomes, microparticles, nanoparticles) are promising formulations for (i) preventing drug degradation rates, (ii) targeting drug to specific anatomical sites associated to bacteria, (iii) improving drug release, (iv) sustaining bactericidal action, and (v) minimizing drug related side-effects (e.g. skin irritation, dryness, allergy). In nanoparticles, the drug is dissolved, entrapped, encapsulated or attached to a matrix composed of natural or synthetic polymers, solid lipids or inorganic materials forming 10–1000 nm drug carriers (Constant et al., 2006).

From the range of active chemical agents ("biocides") used for antisepsis, disinfection and preservation (McDonnell and Russell, 1999), chlorhexidine which has broad-spectrum antibacterial activity against Gram positive and Gram negative bacteria (Aly and Maibach, 1979), has been considered the most acceptable for reducing nosocomial transmission of infections in intensive care units (Frantz et al., 1997). Recently, we showed that chlorhexidine-loaded nanocapsules, exhibiting an immediate and long-lasting antimicrobial activity against (i) several bacterial strains *in vitro*, and (ii) *Staphylococcus epidermidis* inoculated artificially onto skin surface for 8 h, might constitute a promising strategy for lowering the frequency of disinfection (Lboutounne et al., 2002). The sustained antiseptic effect was attributed to the severe adsorption of positively charged nanocapsules to the cell wall of bacteria, and the subsequent chlorhexidine diffusion through the polymer and the bacterial membrane. Furthermore, the small size of such colloidal carriers was consistent with a migration along the pilo-sebaceous units (Lboutounne et al., 2004).

In the present study, an original chlorhexidine-loaded nanocapsule-based gel (Nanochlorex® Pirot, 2006) designed for hygienic hand disinfection was formulated, then tested against resident hand flora under practical conditions in comparison with 2-propanol 60% (v/v). Furthermore, the sustained anti-microbial effect of Nanochlorex® against resident and transient skin flora was compared, in two complementary procedures, with that of a commercial 62% (v/v) ethanol-based hand rub gel (Purell®).

2. Materials and methods

2.1. Chemicals

Chlorhexidine base, poly(ε-caprolactone) (PCL, MW: 42,500 g mol^{-1}), phosphate buffer saline tablets, Triton®-X 100, sodium thiosulfate and sodium thioglycolate were purchased from Sigma–Aldrich (St. Quentin Fallavier, France). Acetone, acetonitrile, sodium acetate, acetic acid and 2-propanol were provided by Prolabo (Lyon, France). Phospholipids (Epikuron® 200) were obtained from Lucas Meyer (St. Maur-des-Fossés, France). Epikuron® 200 is a waxy, purified (minimum 92%) preparation of phosphatidylcholine with accompanying phospholipids (lysophosphatidylcholine maximum 3%, other phospholipids maximum 2%). The total content of fatty acids in Epikuron® 200 was approximately 63%. A nonionic surfactant (polyoxyethylene 20 sorbitan monooleate, Tween® 80) was a gift from ICI surfactant (Sceaux, France). Labrafac hydrophile WL 1219® (caprylic/capric triglyceride PEG-4-esters) was obtained from Gattefossé (St. Priest, France). Sodium salt of carboxymethylcellulose (MW: 550,000 g mol^{-1}, CMC) was purchased from Cooper Rhône-Poulenc Rorer (Melun, France). The degrees of substitution and polymerization of CMC were, respectively 0.7 and 2500 and the sodium content was 7.0–8.9%, calculated on the dry substance. Tryptic soy agar (TSA) and Brewer agar were purchased from VWR International France (Fontenay-sous-Bois, France) and Merck (Darmstadt, Germany), respectively. Purell® Hand Sanitizer (ethanol 62%, v/v) (GOJO, Milton Keynes, UK) was chosen as commercial ethanol-based hand rub gel.

2.2. Formulation of chlorhexidine-loaded nanocapsule-based gel (Nanochlorex®)

Chlorhexidine-loaded nanocapsules were prepared by interfacial polymer deposition following solvent displacement using the method reported previously (Lboutounne et al., 2002, 2004). Briefly, 125 mg PCL and 50 mg Epikuron® 200 were dissolved in 25 ml acetone supplemented with 100 mg chlorhexidine dissolved in 0.5 ml of Labrafac hydrophile WL 1219® at 40 °C. The resulting organic mixture was poured, under light magnetic stirring, into 50 ml of Tween® 80 aqueous solution (0.15%, w/v). The resulting mixed phase immediately turned milky with bluish opalescence (Tyndall effect) because of the instantaneous formation of nanocapsules. Therefore, the nanocapsule suspension was concentrated to a 10-ml final volume, under reduced pressure (at 45 °C) then chlorhexidine loaded-nanocapsules were suspended in 0.4% (w/w) CMC hydrogel (Nanochlorex®). Chlorhexidine concentration in Nanochlorex®, assayed by high performance liquid chromatography as described previously (Lboutounne et al., 2002, 2004), was ∼0.6% (w/v). Particle profiles such as the *z*-average diameter and the polydispersity index (PI), as well as the zeta potential of nanocapsules were determined by a Zetamaster® (Malvern Instruments Orsay, France) using photon correlation spectroscopy (dispersant refractive index = 1.33, detector angle = 90°, wavelength = 670 nm) and electrophoretic

measurement (sample dielectric constant = 79, cell field = 28 V cm^{-1}). The *z*-average diameter was 285 ± 13 nm and the zeta potential of nanocapsules suspended in distilled water was 34 ± 9 mV.

2.3. Bactericidal activity of Nanochlorex® and 2-propanol against resident skin flora

Nanochlorex® was compared with 2-propanol 60% (v/v) on the hands of five informed volunteers. One milliliter of the test product or 2-propanol 60% (v/v) was applied, using a cross-over design, to one hand, whereas the other hand was untreated. A 30-s rub-in period permitted a complete drying of the test product and 2-propanol 60% (v/v). Therefore, hands were washed in sterile plastic bag filled with 400 ml of liquid broth supplemented by neutralisers (i.e. 1% Tween 80®, 0.1% Triton®-X 100, 0.2% sodium thioglycolate and 0.3% sodium thiosulfate) for 5 min (Gaschen, 1968). Subsequent bacterial counts were determined by serial dilution of 1 ml sampling fluid (i) in TSA containing 1% Tween® 80 and (ii) in Brewer anaerobic agar containing 1% Tween® 80 (Eisgruber and Reuter, 1995). Dishes were incubated for 48 h or 8 days at 35 °C in aerobic or anaerobic conditions, respectively, and finally, the colony forming units (CFUs) were counted. For both Nanochlorex® and 2-propanol 60% (v/v), the $\log_{10}$ counts from hands of each subject were separately averaged for pre-values and post-values. The difference between the pre-value and the post-value was the individual $\log_{10}$ reduction factor (RF) (Kampf and Ostermeyer, 2004).

2.4. Bactericidal activity of Nanochlorex® and ethanol-based gel (Purell®) against resident and transient skin flora

2.4.1. Resident skin flora

Eight informed volunteers were treated with both Nanochlorex® and 62% (v/v) ethanol-based gel (Purell®). A rest period of 1 week elapsed between baseline bacterial evaluation and each application of product in order to allow the reconstitution of normal skin flora (Kampf et al., 2005). For the rubbing procedure, 3 ml of Nanochlorex® or Purell® were vigorously rubbed over the hands and fingers up to the wrist until complete drying (30 s). To perform the bacterial sampling technique, loosely fitting powder-free surgical gloves were placed over the hands to be sampled, then, 50 ml of sampling solution (1% Tween® 80, 0.1% Triton®-X 100, 0.2% sodium thioglycolate and 0.3% sodium thiosulfate) were aseptically added to one glove (immediate effect), whereas the other glove was sampled after 3 h (sustained effect) (Grabsch et al., 2004; Mulberrry et al., 2001). Therefore, the gloved hand was uniformly massaged for 1 min, then an aliquot of the sampling solution in the gloves was aseptically transferred to a serial dilution tube containing suitable anti-microbial neutralizers to achieve 1:10 and $1:10^3$ dilutions. One milliliter of each dilution was incorporated in TSA containing 1% Tween® 80 and incubated for 48 h at 35 °C. Efficacy measurements were made on the basis of immediate and sustained anti-microbial effectiveness as determined by the individual RF at post-rub sampling times of 30 s and 3 h.

2.4.2. Transient skin flora

The recently developed *ex vivo* test was adapted for testing the antibacterial activity of Nanochlorex® against *S. epidermidis* on human skin (Lboutounne et al., 2002; Moulari et al., 2005). Bacteria isolated from clinical specimens were suspended in phosphate buffer saline pH 7.4 (PBS) to the concentration of 1.5×10^8 bacteria/ml, corresponding to 0.5 McFarland's standard determined by using ATB 1550 Densiometer® (bioMerieux, France), then, were diluted in PBS to 1.5×10^7 bacteria/ml. Human skin specimens were obtained from abdominal plastic surgery and stored at −20 °C until use for 2 weeks. After thawing, subcutaneous fat was carefully trimmed and the skin specimens (0.7 cm^2) were mounted onto vertical diffusion cells filled with 10 ml of saline solution (NaCl 0.9%) in the receptor compartment. Stratum corneum (SC) surface always faced up the donor compartment. Then 5 μl of either Nanochlorex® or Purell® were applied, with a sterile pipette, onto the delimited skin area for 5 min. One milliliter of *S. epidermidis* inocula (1.5×10^7 CFU/ml) was added to the SC surface. To evaluate the effectiveness of residual hand-gel, inocula were withdrawn at regular time intervals (1, 2, 3 and 4 h) and replaced by fresh *S. epidermidis* suspension onto the SC surface (1, 2 and 3 h). Therefore, inocula collected from SC surface were diluted from $1:10^2$ to $1:10^5$ in PBS containing neutralizers (1% Tween 80®, 0.1% Triton X100, 0.2% sodium thioglycolate and 0.3% sodium thiosulfate in PBS), and were subsequently incorporated into TSA containing 1% Tween® 80. The plates were incubated for 48 h at 35 °C, and the CFUs were visually counted. The survival rates of bacteria were expressed as CFU/ml and transformed into a decimal logarithm. If no CFUs were counted in any of the agar plates, this count was recorded as $<1 \log_{10}$.

2.4.3. Nanocapsule targeting to bacteria

The localization of nanocapsules and skin-associated bacteria in the skin structures was performed by scanning electronic microscopy (SEM) (Lboutounne et al., 2002). Briefly, porcine ear skin samples treated by Nanochlorex® for 30 min were treated with a mixture of 5% glutaraldehyde and phosphate buffer solution (pH 7.3) (1:1, v/v) for 48 h at room temperature. Then, porcine ear skin samples were washed twice with a phosphate buffer solution, dehydrated in acetone and freeze-dried. The freeze-dried porcine ear skin samples were coated with gold–palladium. Using a S800 scanning electron microscope (Hitachi), nanocapsule localization on the SC surface and on skin-associated bacteria was studied.

2.5. Statistical analysis

Student's *t* test was used to establish the significance of the $\log_{10}$ bacterial reduction from baseline (Faoagali et al., 1995), and to compare RF and $\log_{10}$ surviving bacteria determined for each product (i.e. Nanochlorex®, 2-propanol 60% (v/v), Purell®). The chosen level of significance was $P < 0.05$.

Table 1
Immediate efficacy of Nanochlorex® and 2-propanol on resident skin flora after a 30-s hand rub

Formulations	Procedure	Volunteers (*N*)	Aerobic bacteria			Anaerobic bacteria		
			(a) Pre-values (log_{10})	(b) Post-values (log_{10})	RF (a − b)	(a) Pre-values (log_{10})	(b) Post-values (log_{10})	RF (a − b)
2-propanol (60%, v/v)	Handrub	5	6.64 ± 0.23	6.26 ± 0.32*	0.38 ± 0.55	6.74 ± 0.17	6.62 ± 0.22^{NS}	0.12 ± 0.39
Nanochlorex®	Handrub	5	6.63 ± 0.21	6.33 ± 0.14*	0.30 ± 0.35	6.67 ± 0.13	6.42 ± 0.19***	0.25 ± 0.32

Each data is the mean ± standard deviation of five experimental determinations; $^{*}P<0.05$ as compared to "pre-values" group (*t*-test); $^{***}P<0.001$ as compared to "pre-values" group (*t*-test); NS: not significantly different from "pre-values" group (*t*-test).

3. Results

In the present study, an original chlorhexidine-loaded nanocapsule-based gel (Nanochlorex®) was formulated and tested against resident skin flora. Immediate and sustained antibacterial properties of Nanochlorex® were compared with 2-propanol 60% (v/v) and 62% (v/v) ethanol-based gel. Immediate efficacy of Nanochlorex® on resident skin flora was compared to that experienced with 2-propanol 60% (v/v), after a 30-s hand rub. Both formulations reduced post-values of surviving aerobic bacteria on hands; Nanochlorex® reduced bacteria by an average log_{10} reduction factor, which was not found significantly different from the one determined with 2-propanol 60% (v/v) (0.30 versus 0.38) (Table 1). However, a 30-s hand rub with 2-propanol 60% (v/v) was not found effective against anaerobic bacteria, whereas Nanochlorex® achieved the required efficacy.

By using the glove juice technique, the sustained efficacy of Nanochlorex® on resident skin flora was compared with 62% (v/v) ethanol-based gel (Purell®), after 30 s and 3 h. As reported in Table 2, the immediate antibacterial efficacy of Nanochlorex® was not significantly different from the one determined with Purell® (RF: 0.55 versus 0.44, $P>0.05$). However, the hand rub realized with Purell® was not found effective to insure a significant decrease of bacterial post-values at 3 h. Nevertheless, a sustained efficacy of Nanochlorex® was shown against bacteria as evidenced by the comparable average log_{10} reduction factors determined at 30 s and 3 h ($RF_{1\ min}$: 0.44; $RF_{3\ h}$: 0.60).

The *ex vivo* test used for investigating the sustained activity of Nanochlorex® involved repeated contaminations of human skin by *S. epidermidis*. Fig. 1 showed the surviving bacteria counts (expressed as log_{10} reduction of CFU/ml) collected from *ex vivo* human skin specimens initially treated by either Nanochlorex® or 62% (v/v) ethanol-based gel (Purell®) for 5 min, then artificially contaminated at time +5 min, +1 h, +2 h, and +3 h. A significant difference in the log_{10} CFU/ml was confirmed between Nanochlorex® and Purell® at time 1 h ($P<0.001$); 2 h ($P<0.001$); 3 h ($P<0.01$). As depicted, only Nanochlorex® exhibited a sustained effect against bacteria after repeated contaminations (~5.5 log_{10} reduction after the first contamination; ~2.5 log_{10} reduction after the second contamination; ~1.5 log_{10}

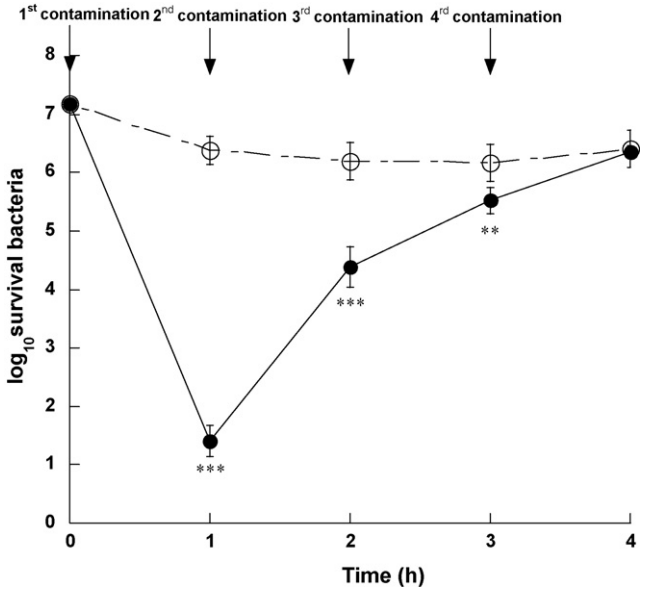

Fig. 1. Comparison of sustained antibacterial effect of (●) Nanochlorex® and (○) Purell® applied to human skin for 5 min, then contaminated successively by *Staphylococcus epidermidis* at time +5 min, +1 h, +2 h, and +3 h. Each data is the mean ± standard deviation of at least four experimental determinations. $^{**}P<0.01$ as compared to Purell® group (*t*-test); $^{***}P<0.0001$ as compared to Purell® group (*t*-test).

Table 2
Bactericidal efficacy of Nanochlorex® in comparison with ethanol-based gel (Purell®) on resident skin flora after 30 s hand rub

Formulations	Procedure	Volunteers (*N*)	Immediate effect (30 s)			Sustained effect (3 h)		
			(a) Pre-values (log_{10})	(b) Post-values (log_{10})	RF (a − b)	(a) Pre-values (log_{10})	(b) Post-values (log_{10})	RF (a − b)
62% ethanol-based gel (Purell®) (v/v)	Handrub	8	6.39 ± 0.32	5.84 ± 0.48**	0.55 ± 0.40	6.37 ± 0.30	6.08 ± 0.37^{NS}	0.29 ± 0.33
Nanochlorex®	Handrub	8	6.39 ± 0.32	5.95 ± 0.39***	0.44 ± 0.20	6.37 ± 0.30	5.77 ± 0.23*	0.60 ± 0.36$^{¥}$

Each data is the mean ± standard deviation of eight experimental determinations; $^{*}P<0.05$ as compared to "pre-values" group (*t*-test); $^{**}P<0.01$ as compared to "pre-values" group (*t*-test); $^{***}P<0.001$ as compared to "pre-values" group (*t*-test); NS: not significantly different from "pre-values" group (*t*-test); $^{¥}P<0.05$ as compared to Purell® group (*t*-test).

reduction after the third contamination). In contrast, Purell® showed only ∼1 $\log_{10}$ reduction factor throughout the experimental period. After the fourth contamination, no significant difference in antibacterial activity was shown between both products.

4. Discussion

Over the world, nosocomial infections constitute a serious threat for patients (e.g. 2 million cases reported annually in the United States (Pittet and Donaldson, 2005)), involving a resurgence of hand hygiene in the healthcare systems (Kampf and Ostermeyer, 2003). In this field, the importance of hygienic hand disinfection with "leave-on" products has been recently recognized by the World Health Organisation Alliance for Patient Safety launching the first biennial Global Patient Safety Challenge, entitled "clean care is safer care", which targets infections associated with health care (Pittet and Donaldson, 2005; WHO, 2006). Therefore, the hygienic hand disinfection products (mainly alcohol-based hand rubs) claim (i) to reduce or inhibit the growth of microorganisms with maximum efficacy, a fast acting and broad-spectrum activity, some excellent microbiocidal characteristics, a lack of potential emergence of resistance, (ii) to overcome the lack of accessibility to sinks or other facilities (including clean running water or towels in some poor and remote areas), (iii) to perform hand cleansing actions that require the use of water (hand washing and hand antisepsis using a formulation different from a waterless agent), (iv) to improve compliance with hand hygiene by reducing the time required to perform it and the convenience of the method, and (v) to reduce costs (WHO, 2006).

In spite of those advantages, alcohol-based hand rubs exhibit some drawbacks: a rapid evaporation of alcohol compromising sustained bactericidal effect; skin dryness caused or maintained by repeated exposure to alcohol (Harbarth et al., 2002); impairment of efficacy due to the persistence of skin care products or emollient used for limiting skin dryness (Boyce and Pittet, 2002).

Chlorhexidine is a widely used antiseptic product, characterized by a broad-spectrum of efficacy, cutaneous remanence and low irritant properties (McDonnell and Russell, 1999). In a previous study, chlorhexidine base entrapped within nanocapsules showed a sustained bactericidal activity against *S. epidermidis* inoculated onto porcine ear skin (Lboutounne et al., 2002). In the present study, the potential of Nanochlorex® to provide immediate and sustained antibacterial activity was investigated *in vivo* and *ex vivo* on human skin, by comparison with 2-propanol 60% (v/v) and 62% (v/v) ethanol-based gel (Purell®).

Although hand washing is a part of hygienic hand disinfection, it was excluded from the present technical procedure because the hand wash may impair the efficacy of the consecutive hand rub and promote skin dryness and dermal irritation (Labadie et al., 2002).

As reported previously, the immediate antibacterial effect of Nanochlorex® might be explained by (i) the rapid desorption of chlorhexidine from the nanocapsule wall and (ii) the subsequent diffusion within bacteria; whereas the sustained antibacterial effect would be the consequence of a slow release of chlorhexidine from the nanocapsule core against further bacterial colonization (Lboutounne et al., 2002, 2004). These assumptions were confirmed by nanocapsule adhesion to SC surface and SC-associated bacteria wall which target chlorhexidine to specific bacterial structures and lengthen the antibacterial activity, as evidenced in Figs. 2 and 3. Re-colonization of skin surface by bacteria was preserved at low rate by the gelling agent (i.e. CMC), which reduced water available for bacteria growth by osmotic shock, and maintained a thickening system on SC acting as a physical barrier against bacterial influx. In the present study, the user-acceptability of Nanochlorex® was confirmed by users which noted no tackiness, crumbly skin feeling or discomfort in wearing gloves during the investigation.

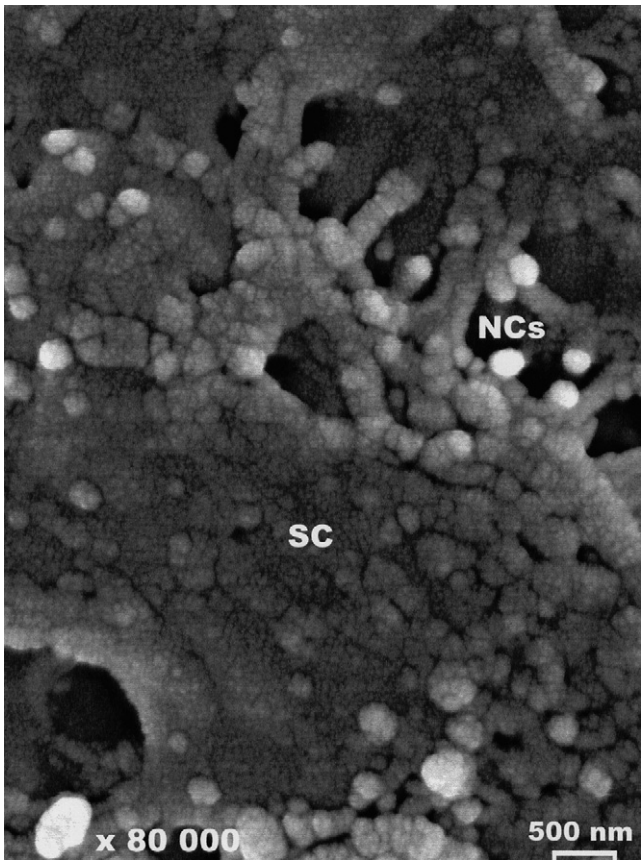

Fig. 2. Scanning electron micrographs of chlorhexidine-loaded nanocapsules (NCs) localization on porcine stratum corneum (SC) treated by Nanochlorex® for 30 min (80,000×, bar: 500 nm).

In many European countries alcohol (i.e. ethanol, 1-propanol, 2-propanol)-based liquid products have been established as a standard in hygienic hand disinfection (Kampf and Ostermeyer, 2004); whereas a recent study showed a limited efficacy of alcohol-based hand gel (Kramer et al., 2002). Our data showed that Nanochlorex® was (i) as effective as 2-propanol 60% (v/v) and 62% (v/v)ethanol-based hand gel (Purell®) within 30 s of application; furthermore, Nanochlorex® proved to be more efficient than 2-propanol 60% (v/v) against anaerobic bacteria and

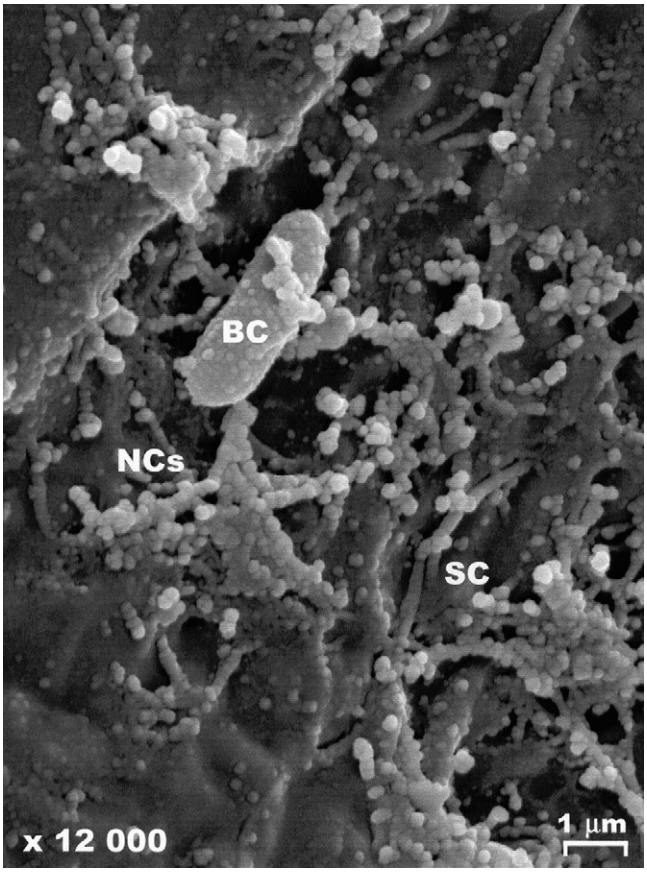

Fig. 3. Scanning electron micrographs of chlorhexidine-loaded nanocapsules (NCs) localization on (i) stratum corneum (SC) treated by Nanochlorex® for 30 min and (ii) on SC-associated bacteria (BC) (12,000×, bar: 1 μm).

exhibited a long-lasting antibacterial activity higher than that of Purell®. The lower efficacy of alcohol-based hand gel was attributed to the high evaporation rate of ethanol incompatible with satisfactory sustaining antibacterial effect (Bush et al., 1986).

5. Conclusion

In the present study, we confirmed the potent antibacterial activity of chlorhexidine-loaded nanocapsule-based gel (Nanochlorex®) against resident and transient skin flora. The results of these in-use tests showed that Nanochlorex® had bactericidal efficacy similar to 2-propanol 60% (v/v) after a 30-s hand rub, but exhibited superior antibacterial and residual effect compared to 62% (v/v) ethanol-based hand gel. Therefore, the suitability of Nanochlorex® for skin antisepsis has been demonstrated as a promising approach in care practice in which numerous procedures have to be respected.

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References

Aly, R., Maibach, H.I., 1979. Comparative study on the antimicrobial effect of 0.5% chlorhexidine gluconate and 70% isopropyl alcohol on the normal flora of hands. Appl. Environ. Microb. 37, 610–613.

Boyce, J.M., Pittet, D., 2002. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Infect. Control Hosp. Epidemiol. 23, S3–S40.

Bush, L.W., Benson, L.M., White, J.H., 1986. Pig skin as test substrate for evaluating topical antimicrobial activity. J. Clin. Microbiol 24, 343–348.

Constant, H., Falson, F., Pirot, F., 2006. Current and new strategies for the delivery of antiseptic agents. Curr. Drug Deliv. 3, 315–323.

Eisgruber, H., Reuter, G., 1995. A selective medium for the detection and enumeration of mesophilic sulphite-reducing clostridia in food monitoring programs. Food Res. Int. 28, 219–226.

Faoagali, J., Fong, J., George, N., Mahoney, P., O'Rourke, V., 1995. Comparison of the immediate, residual, and cumulative antibacterial effects of Novaderm R, Novascrub R, Betadine Surgical Scrub, Hibiclens, and liquid soap. Am. J. Infect. Control 23, 337–343.

Frantz, S.W., Haines, K.A., Azar, C.G., Ward, J.I., Homan, S.M., Roberts, R.B., 1997. Chlorhexidine gluconate (CHG) activity against clinical isolates of vancomycin-resistant Enterococcus faecium (VREF) and the effects of moisturizing agents on CHG residue accumulation on the skin. J. Hosp. Infect. 37, 157–164.

Gaschen, M., 1968. Comparative study on 2 methods for sampling the microbial flora of hands. Helv. Chir. Acta 35, 372–377.

Grabsch, E.A., Mitchell, D.J., Hooper, J., Turnidge, J.D., 2004. In-use efficacy of a chlorhexidine in alcohol surgical rub: a comparative study. ANZ J. Surg. 74, 769–772.

Harbarth, S., Pittet, D., Grady, L., Zawacki, A., Potter-Bynoe, G., Samore, M.H., Goldmann, D.A., 2002. Interventional study to evaluate the impact of an alcohol-based hand gel in improving hand hygiene compliance. Pediatr. Infect. Dis. J. 21, 489–495.

Kampf, G., Jarosch, R., Ruden, H., 1998. Limited effectiveness of chlorhexidine based hand disinfectants against methicillin-resistant *Staphylococcus aureus* (MRSA). J. Hosp. Infect. 38, 297–303.

Kampf, G., Kramer, A., 2004. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin. Microbiol. Rev. 17, 863–893.

Kampf, G., Ostermeyer, C., 2003. Inter-laboratory reproducibility of the hand disinfection reference procedure of EN 1500. J. Hosp. Infect. 53, 304–306.

Kampf, G., Ostermeyer, C., 2004. Efficacy of alcohol-based gels compared with simple hand wash and hygienic hand disinfection. J. Hosp. Infect. 56, 13–15.

Kampf, G., Ostermeyer, C., Heeg, P., 2005. Surgical hand disinfection with a propanol-based hand rub: equivalence of shorter application times. J. Hosp. Infect. 59, 304–310.

Kramer, A., Rudolph, P., Kampf, G., Pittet, D., 2002. Limited efficacy of alcohol-based hand gels. Lancet 359, 1489–1490.

Labadie, J.C., Kampf, G., Lejeune, B., Exner, M., Cottron, O., Girard, R., Orlick, M., Goetz, M.L., Darbord, J.-C., Kramer, et al., 2002. Recommendations for surgical hand disinfection—requirements, implementation and need for research. A proposal by representatives of the SFHH, DGHM and DGKH for a European discussion. J. Hosp. Infect. 51, 312–315.

Lboutounne, H., Chaulet, J.-F., Ploton, C., Falson, F., Pirot, F., 2002. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(epsilon-caprolactone) nanocapsule encapsulated form and as a digluconate. J. Control. Release 82, 319–334.

Lboutounne, H., Faivre, V., Falson, F., Pirot, F., 2004. Characterization of transport of chlorhexidine-loaded nanocapsules through hairless and wistar rat skin. Skin Pharmacol. 17, 176–182.

Maillard, J.Y., Messager, S., Veillon, R., 1998. Antimicrobial efficacy of biocides tested on skin using an ex-vivo test. J. Hosp. Infect. 40, 313–323.

McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 12, 147–179.

Messager, S., Goddard, P.A., Dettmar, P.W., Maillard, J.Y., 2001. Determination of the antibacterial efficacy of several antiseptics tested on skin by an 'ex-vivo' test. J. Med. Microbiol. 50, 284–292.

Moulari, B., Lboutounne, H., Pellequer, Y., Guillaume, Y.C., Millet, J., Pirot, F., 2005. Vectorization of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae) ethanolic leaf extract by using PLG-nanoparticles: antibacterial activity assessment. Drug Dev. Res. 65, 26–33.

Mulberrry, G., Snyder, A.T., Heilman, J., Pyrek, J., Stahl, J., 2001. Evaluation of a waterless, scrubless chlorhexidine gluconate/ethanol surgical scrub for antimicrobial efficacy. Am. J. Infect. Control 29, 377–382.

Pirot, F., 2006. Nanochlorex (no. 06/3404615). Institut National de la Propriété Industrielle, Lyon, France.

Pittet, D., Donaldson, L., 2005. Clean care is safer care: a worldwide priority. Lancet 366, 1246–1247.

Rotter, M.L., 2004. European norms in hand hygiene. J. Hosp. Infect. 56, 6–9.

WHO, 2006. Global Patient Safety Challenge of the World Alliance for Patient Safety. World Health Organization, Geneva, Switzerland http://www.who.int/patientsafety/challenge/en/ (accessed September 18, 2005).