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Skin Healthcare by Innovative NanoCAPsules

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Deliverable D21

D3.6 Lotions and textiles for skin antimicrobial care containing the targeted nanocapsules

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Abstract	A natural essential oil was used for the preparation of nanocapsules (NCs) for antimicrobial applications. The NCs were fully characterised and their antimicrobial properties were evaluated <i>in-vivo</i> and <i>in-vitro</i> . The results revealed that the incorporation of the essential oil into sustainable and bio-based stable NCs provided antimicrobial activity against several microorganisms.
File Name	SKHINCAPS_ Deliverable D3.6 Lotions and textiles for skin antimicrobial care containing the targeted nanocapsules

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INTRODUCTION

Nowadays, the increased awareness of consumers towards healthy and sustainable lifestyle habits is leading to the continuous seek for new technologies suitable for diverse industry sectors, that could satisfy and fulfil people's needs. In this context, nanotechnology is playing a crucial role in the creation of added value daily products, mainly because of the great properties achieved at nanoscale.

Skin Healthcare by Innovative NanoCAPsules project aimed to develop customised products (cosmetics and textiles) for skin care using an innovative, cost-effective, safe and sustainable *in situ* self-assembly nanoencapsulation technology, based on biocompatible and biodegradable polymers and natural active ingredients. These nanocapsules/nanocarriers are stimuli-responsive, safe, controllable and have different release mechanisms for the active ingredients, to achieve distinct properties: thermal comfort, anti-ageing and antimicrobial.

In the case of antimicrobial products, nanocapsules (NCs) with a targeted release mechanism and containing natural antimicrobial compounds (essential oils, EO) were developed for incorporation into several creams (anti-acne, body gel, athlete's foot and anti-dandruff shampoo) and textiles (Figure 1).



Figure 1. Skin anti-ageing demonstrators (creams and sweater).

The grafting of selected antibodies onto the shell of the essential oil-loaded nanocapsules allowed the control of skin Gram-positive bacterial infections (e.g. in atopic dermatitis conditions) by means of a target release mechanism. The obtained nanocapsules were able to reduce the growth of the target Gram-positive bacteria *S. aureus* but showed no effect on other bacterial cells or on human skin cells.

The results from Figure 2 confirm that the formulated antimicrobial nanocapsules selectively reduced the growth of the Gram-positive *S. aureus* but had only a slight reduction on the growth of *P. aeruginosa*, due to the presence of the specific ligand that drives the antibacterial essential oil nanocapsules directly to the target bacteria. In contrast, essential oil nanocapsules without the targeting element showed bactericidal activity towards both bacteria – the Gram-positive *S. aureus* and Gram-negative *P. aeruginosa*.

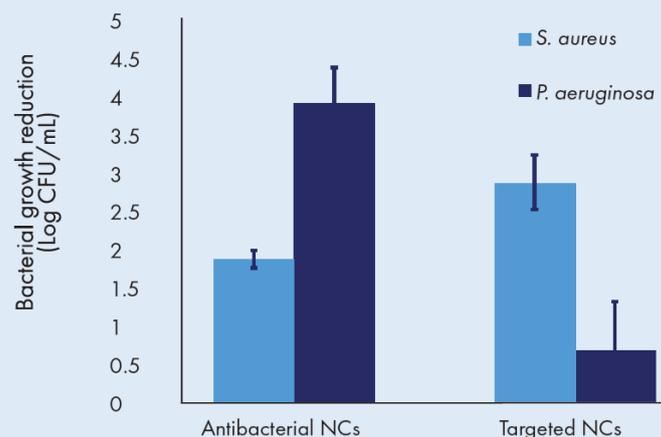


Figure 2. *S. aureus* and *P. aeruginosa* growth reduction upon incubation with antibody-immobilized NCs and non-targeted EO-loaded NCs. The NCs were incubated with both *S. aureus* and *P. aeruginosa* for 24h.

ANTIMICROBIAL COSMETICS

The essential oil-loaded nanocapsules have shown to reduce the *S. aureus* viable cells, which means they possess bactericidal effect, and were further used for the preparation of different antibacterial cosmetic formulations, namely anti-acne cream and body gel.

The **anti-acne cream** developed on SKHINCAPS was tested against Gram-positive *P. acnes*, which is considered one of the main bacteria responsible for acne development (Figure 3). The inclusion of essential oil-nanocapsules into the anti-acne formulations increased the killing efficiency compared to the standard cream (control anti-acne formulation without essential oil) against the targeted bacteria.

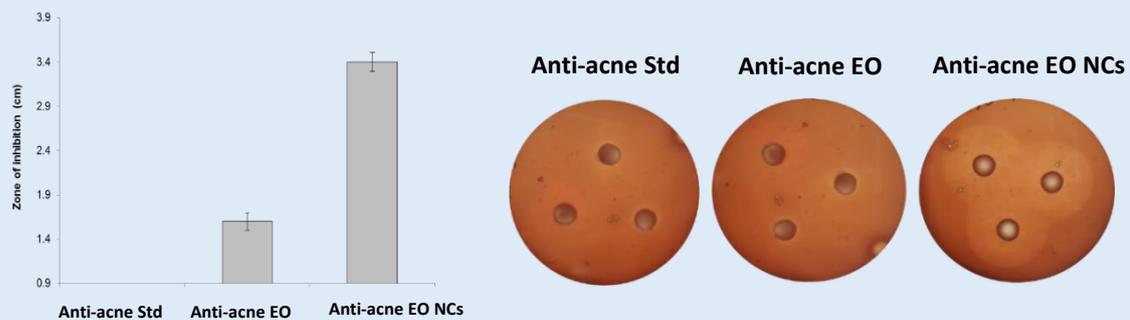


Figure 3. Antibacterial efficacy of anti-acne creams containing EO-loaded NCs on *P. acnes*. The samples were incubated in an agar plate previously loaded with *P. acnes* (CFU/mL $\sim 10^5$).

These formulations were also tested against the Gram-positive *S. aureus* (Figure 4), whereas the inclusion of essential oil-nanocapsules into the cream increased its bactericidal efficacy.

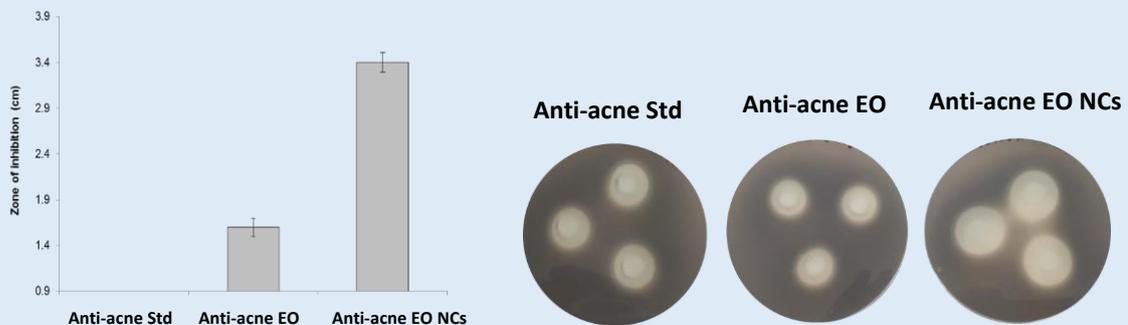


Figure 4. Antibacterial efficacy of anti-acne creams containing EO-loaded NCs on *S. aureus*. The samples were incubated in an agar plate previously loaded with *S. aureus* (CFU/mL $\sim 10^5$).

The antibacterial efficacy of the developed **body gel** formulation against both *S. aureus* and *P. acnes* was also assessed (Figure 5). The results revealed that the incorporation of essential oil-loaded nanocapsules on the formulation has improved the antibacterial activity towards *S. aureus* by 30 %.

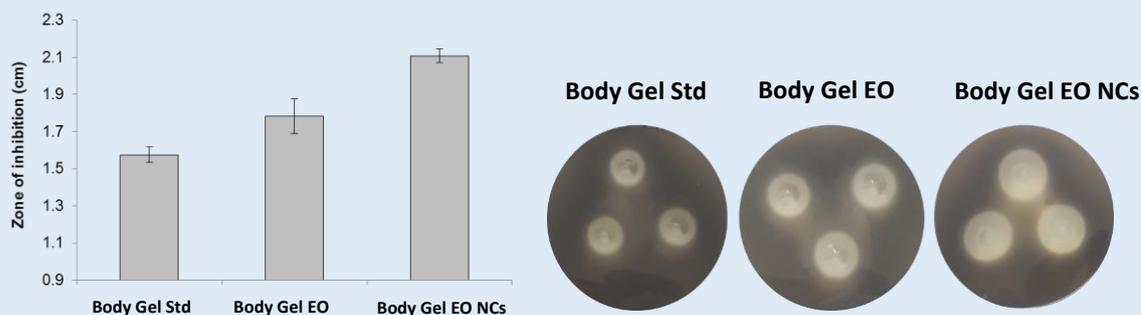


Figure 5. Antibacterial efficacy of body gel loaded with EO and EO NCs. The samples were incubated in an agar plate previously loaded with *S. aureus* (CFU/mL $\sim 10^5$).

Similar tendency was obtained for *P. acnes* - the inclusion of the essential oil-loaded nanocapsules into the body gel formulation led to an improved bactericidal effect (Figure 6).

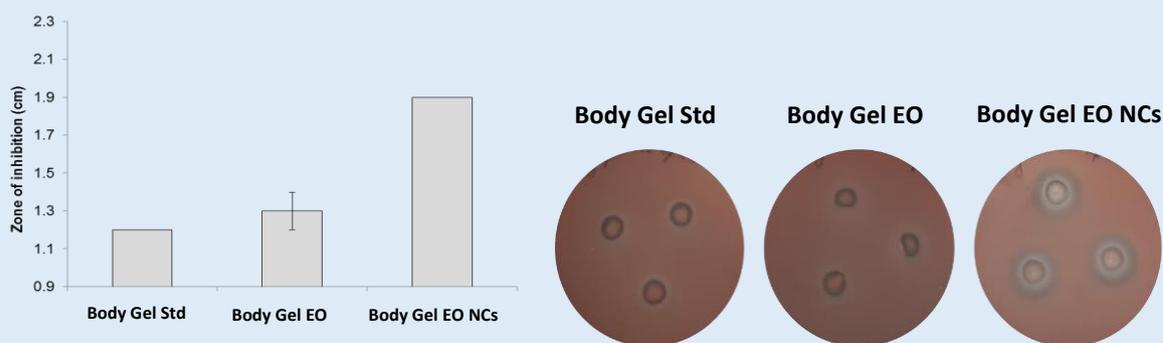


Figure 6. Antibacterial efficacy of body gel loaded with EO and EO-loaded NCs. The samples were incubated in an agar plate previously loaded with *P. acnes* (CFU/mL $\sim 10^5$).

The antifungal activity of the **body gel** was also evaluated. For this purpose, *Trichophyton rubrum* was selected due to its prevalence in athlete's foot infection and the possible application of this gel to avoid the fungal colonization.

The different antimicrobial formulations were also submitted to several tests to obtain information about their stability and safety regarding skin application (Table 1). From the results obtained under the adopted experimental conditions for the investigated demonstrators, they may be judged, overall as VERY GOOD.

Table 1. Preliminary test performed to evaluate the stability and safety regarding skin application of the anti-acne cream and the body gel formulations.

TEST	Anti-acne cream	Body Gel
Accelerate ageing stability study	OK	OK
Microbiological control according 1223/2009	OK	OK
Patch test (Skin irritation)	OK	OK
Challenge (preservative efficacy)	OK	OK
Efficacy against <i>P. acnes</i>	OK	OK
Efficacy against <i>S. aureus</i>	OK	OK
Efficacy against <i>T. rubrum</i>	OK	OK
Moisturizing effect	T30:147%	T30: 154%
Irritation eye	OK	OK
Transdermal Epidermal Water Loss (TEWL)	OK	OK

The efficacy of the developed demonstrators was also evaluated by *in-vivo* studies. For the **anti-acne cream**, the use test aims to verify if the product complies with the following properties:

- The product provides smooth skin;
- The product helps to reduce visibility of pimples and blemishes;
- The product provides a clear face;
- The product clears the impurities of the skin;
- The product provides soft skin;
- The product moisturizes the skin;
- The product reduces skin irritation;

- The product provides comfortable skin care.

These *in-vivo* studies were conducted by the application of the cream in the face of 25 healthy subjects (men and women over 18 years and with oily and combination skin prone to acne) that have used the products for 28 consecutive days (Figure 7). All the tests were performed under dermatological control and a signed consent agreement. Under these study conditions, after 28 days of regular application it is concluded that the anti-acne demonstrator:

- ✓ was very well tolerated at the application site;
- ✓ properties declared by the Customer have been confirmed;
- ✓ reduces the secretion of sebum (average 22%).

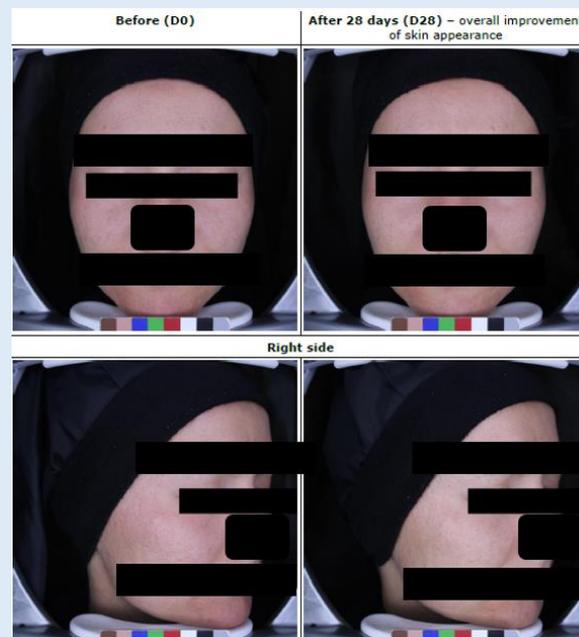


Figure 7. Photo before and after product application of the developed anti-acne cream.

For the **body-gel** formulation, apart from the antimicrobial efficacy against *P. acnes* and *S. aureus*, it was also necessary to evaluate its moisturizing effect, an important property that can typically be observed in this type of product. The efficiency of the body gel was tested on 10 healthy women (between 18-70 years old and with dry skin) after a single cutaneous application. The moisturizing effect on the upper layers of the epidermis was then evaluated by corneometry (skin electrical capacitance measurements). Under these test conditions, the single cutaneous application of the investigational product designated as “BODY GEL” has shown a MARKED MOISTURIZING effect of the skin upper layers after 30 minutes, 2 hours, 8 hours and 24 hours of the application (Table 2).

Table 2. Evaluation of the moisturizing effect of the body gel formulation after a single cutaneous application.

Moisturizing improvement at t= 30min	+154%
Moisturizing improvement at t = 2h	+119%
Moisturizing improvement at t = 8h	+48%
Moisturizing improvement at t = 24h	+37%

Although the nanocapsules were developed to have a targeted release profile, their active release in different conditions (triggered release) was also assessed. The test conditions were chosen considering two relevant scenarios:

- At high skin pH (i.e. pH=7) the skin is more sensitive to infections. Specifically, facial skin pH is higher in patients with recurrent acne¹;
- The warm, moist and sweat favours the microbial colonization. Although fungal infections can occur on almost any area of the body, they are more commonly in intertriginous regions and proliferate under moist conditions.

¹ Prakash C, Bhargava P, Tiwari S, Majumdar B, Bhargava RK. Skin Surface pH in Acne Vulgaris: Insights from an Observational Study and Review of the Literature. The Journal of Clinical and Aesthetic Dermatology. 2017;10 (7):33-39.

The results have shown that the developed nanocapsules exhibit a higher release of the antimicrobial essential oil on critical skin conditions, a clear indication that the antimicrobial formulations developed on SKHINCAPS can avoid the microbial colonization when the skin appears to be in fragile conditions.

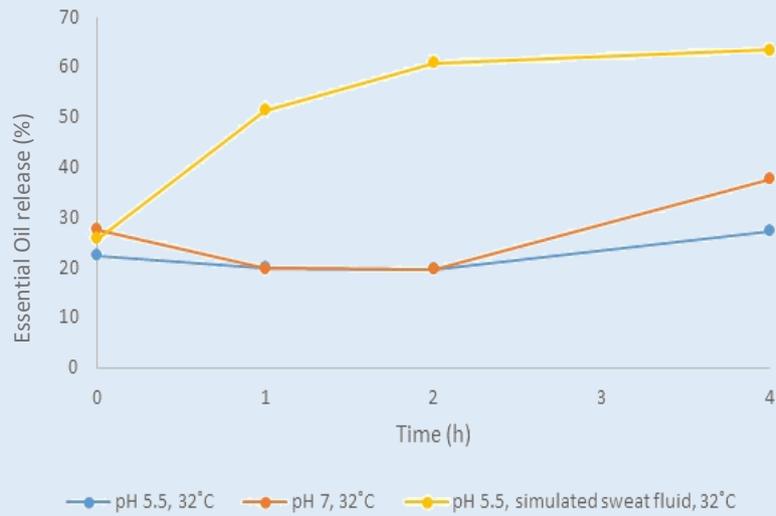


Figure 8. Essential oil released from natural based nanocapsules under relevant skin conditions.

PRODUCT SPECIFICATIONS

Name: Anti-acne cream

PHYSICO-CHEMICAL PROPERTIES:

- ✓ Colour, Odour, Appearance: Pale yellowish opaque. Characteristic odour due to its fragrance.
- ✓ Viscosity (22 °C): 200.000- 400.000 cps
- ✓ pH (22 °C): 6,0 – 7,0



MICROBIOLOGICAL PROPERTIES:

- ✓ Total aerobes: < 100 ufc/ml
- ✓ Moulds and yeasts: < 10 ufc/ml
- ✓ Pathogens (*Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*): Absence

PRODUCT CHARACTERISTICS:

- ✓ Easy application and rapid absorption.
- ✓ Non-greasy.
- ✓ Helps to reduce visibility of pimples and blemishes.
- ✓ Clears the impurities of the skin.
- ✓ Reduces skin irritation.
- ✓ Provides comfortable skin care.
- ✓ Moisturizes the skin (+45% T8h).
- ✓ Reduces the secretion of sebum (average 22%).

INSTRUCTIONS FOR USE:

- ✓ Apply every day, morning and night, after cleaning your skin, by means of a gentle circular massage.
- ✓ Face care product.
- ✓ Product intended for oily and combination skin prone to acne.

WARNINGS:

- ✓ For external use only.

Name: Body gel

PHYSIO-CHEMICAL PROPERTIES:

- ✓ Colour, Odour, Appearance: Transparent viscous gel green coloured emulsion. Odour due to its fragrance.
- ✓ Viscosity (22 °C): >300.000 cps
- ✓ pH (22 °C): 6,0 – 7,0

MICROBIOLOGICAL PROPERTIES:

- ✓ Total aerobes: < 100 ufc/ml
- ✓ Moulds and yeasts: < 10 ufc/ml
- ✓ Pathogens (*Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*): Absence



PRODUCT CHARACTERISTICS:

- ✓ Aloe Vera Gel 99%. Aloe Vera is an excellent moisturizer which provides water to the skin. It also has humectant and emollient effects, providing freshness and well-being to the skin.
- ✓ Panthenol (provitamin B5) is a skin regenerator involved in the reconstruction of the skin natural barrier.
- ✓ Paraben free.
- ✓ Hydrating body gel.
- ✓ Helps to reduce skin's bacterial activity (inhibition proven against *T. rubrum*, *S. aureus* and *P. acne*).

INSTRUCTIONS FOR USE:

- ✓ This product can be applied in the entire body.
- ✓ Product intended for all skin types.

WARNINGS:

- ✓ For external use only.
- ✓ Do not swallow.

MARKETING POINT OF VIEW

The use of nanotechnology in cosmetics has acquired prominence due to the enhanced properties attained by the particles at the nano level including colour, transparency, solubility, antimicrobial or antioxidant activity, among others. Consequently, nanocosmetics have forced the cosmetic industries to limit the use of nanotechnology in cosmetics and to enforce laws to undergo a full-fledged safety assessment before they enter into the market.

SKHINCAPS project explored an innovative and sustainable *in situ* self-assembly nanoencapsulation technology to deliver novel products for skin healthcare applications, with increased efficiency and cost benefits, leading to ground-breaking innovations on the actual products. SKHINCAPS project was working with nanocapsules with sizes over 100 nm. Considering the size, the developed nanocapsules would be considered like other current cosmetic ingredients but adding the value of their delivery system and consequently, increased efficiency for skin application.

Historically, oral ketoconazole was shown to be an effective treatment for *T. rubrum* infections but is no longer used for this indication due to the risk of liver damage as a side effect, similar to the use of terbinafine and naftifine creams. Anti-acne treatments might be aggressive for skin, with some side effects (redness, burned skin, peeling, dried skin and photosensitivity).

At SKHINCAPS project, two antimicrobial products were developed:

- Hydrating body gel focused for healthy end consumers and/or with recurrent fungal infections or acne problems;
- Anti-acne facial cream focused in adults with prone skin.

These demonstrators are intended for application to face skin (anti-acne) and body (body gel), having enhanced antimicrobial action, since they are capable of penetrating into the deep layers of the skin, potentiating the effects of the actives. Both products have shown to be more effective than the standard products (without the actives) and presented no side effects comparing to aggressive treatments.

The antimicrobial products developed would be classified at Medium-High quality (lower price than big & famous competitors like Sesderma, Clinique, Avène, La Roche Posay, but the highest quality in the market), being really good value for money.

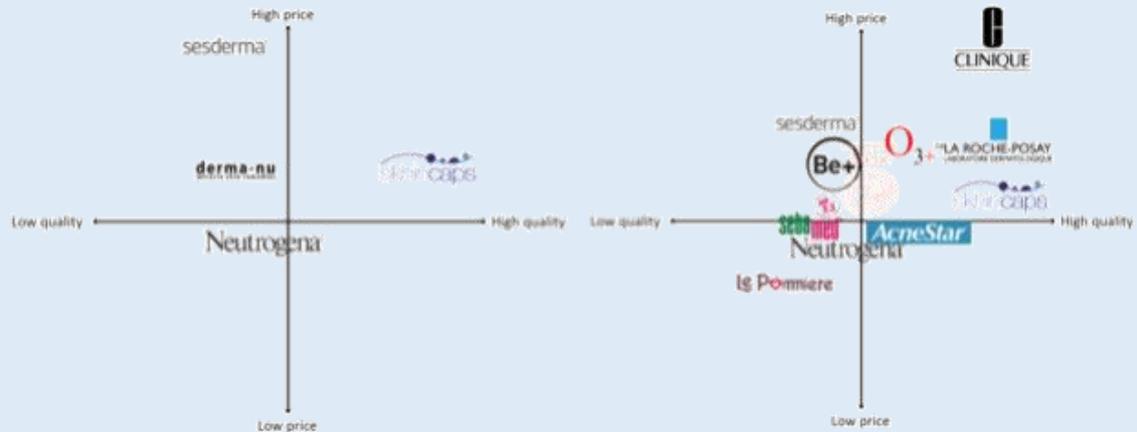


Figure 9. Positioning of body gel (right side) and anti-acne cream (left side) demonstrators developed in SKHINCAPS project.

Based on internal customer relations like visiting distributors and pharmacies with salesforce or by website and social media, TELIC intends to demonstrate the actual benefits of the demonstrators developed to its customers. As soon as all technical and regulatory requirements are performed, and cost-benefit is fully evaluated, the time to market is forecasted to be around 1-year and so TELIC expects significant commercial achievements from these demonstrators' commercialisation.

ANTIMICROBIAL TEXTILES

The innovative essential oil loaded nanocapsules with a targeted release profile were also used for the development of textile structures intended to be used next to skin, the often called first layer textiles, and therefore cotton was the preferred substrate to produce an adult sweater.

The developed demonstrators maintained the loaded nanocapsules even after several washing tests (10 washing cycles performed on a domestic washing machine) and after abrasion tests (500 and 1000 abrasion cycles) that simulated their day-to-day usage (Figure 10). Results were also confirmed by SEM analysis (Figure 11). These promising results are mainly due to the suitable functional groups present in the nanocapsules surface that improved the linkage to textiles, even without using a crosslinker during the functionalization process.

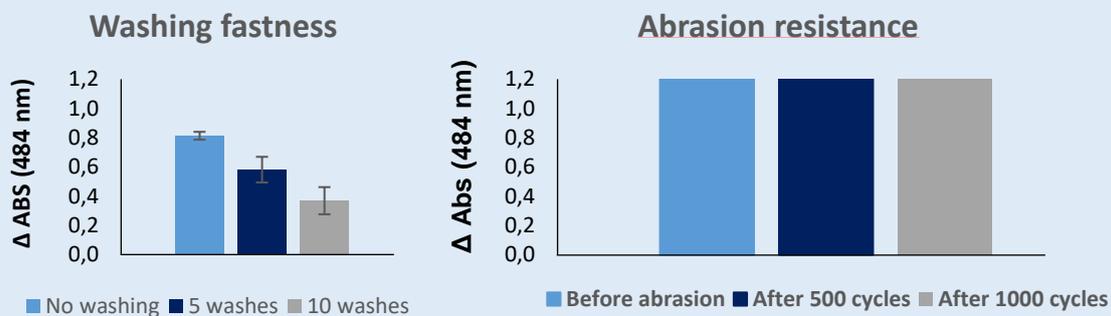


Figure 10. Detection of the protein based antimicrobial NCs on cotton textiles after several washing cycles (left) and after abrasion cycles (right).

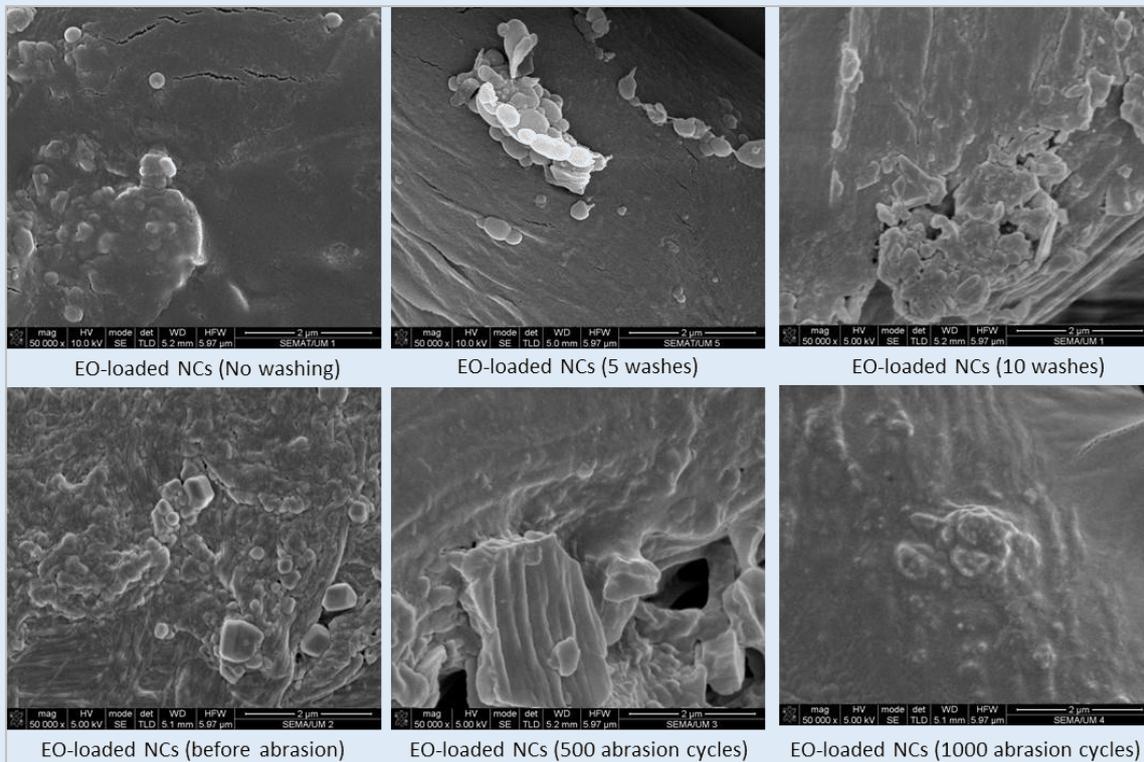


Figure 11. SEM micrographs (50 000 x) of the treated cotton textile with EO-loaded NCs before and after washing (above) and before and after abrasion test (below).

In order to evaluate the antimicrobial properties of the demonstrators, a normative test was performed in a certified laboratory. After 5 washing cycles, the treated textile with essential oil-loaded nanocapsules presented higher antibacterial activity against *S. aureus* than the treated textile with the non-encapsulated essential oil, indicating a good performance of this demonstrator (Figure 12).

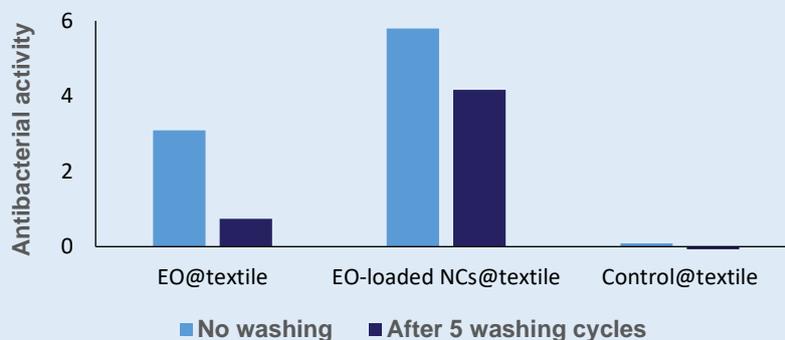


Figure 12. Antibacterial activity (against *S. aureus*) of the control and treated textiles with free and nanoencapsulated EO, before and after 5 washing cycles (ISO 20743).

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SKHINCAPS was committed with the flagships initiatives, and with a number of wider Horizon 2020 objectives including: control healthcare expenditure, Horizon 2020 strategic cosmeceuticals sector and plural Horizon 2020 Key Enabling Technologies (KETs).