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SKIN Healthcare by Innovative NanoCAPsules

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Deliverable D20

D3.4 Creams for skin anti-ageing care containing the triggered nanocapsules

Responsible Partner	Telic
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Abstract	Different natural active principles were used for the preparation of various nanocapsules (NCs) for anti-ageing applications (cosmetics and textiles). The nanocapsules were fully characterised and their antioxidant and anti-wrinkle properties were evaluated <i>in-vivo</i> and <i>in-vitro</i> . The results revealed that their incorporation into sustainable and bio-based stable nanocapsules provided antioxidant activity and anti-wrinkle properties to the demonstrators.
File Name	SKHINCAPS_D3.4 Creams for skin anti-ageing care containing the triggered 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 anti-ageing products, nanocapsules with a triggered release mechanism and containing natural antioxidant compounds were developed for incorporation into creams and textiles (Figure 1).



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

SKIN ANTIAGEING COSMETICS

The anti-ageing efficacy of the developed cosmetics was assessed by *in-vitro* and *in-vivo* tests.

For the *in-vitro* assays, skin fibroblasts cells were used, allowing a screening of the antioxidant activity against reactive oxidative species in a more realistic way than the currently used “test tube” DPPH method.¹ The results showed that the Vitamins nanocapsules and the mixture of Vitamins and essential oil (EO) nanocapsules in the formulations (cream and serum) increased their antioxidant activity compared to the standard products (Figure 2).

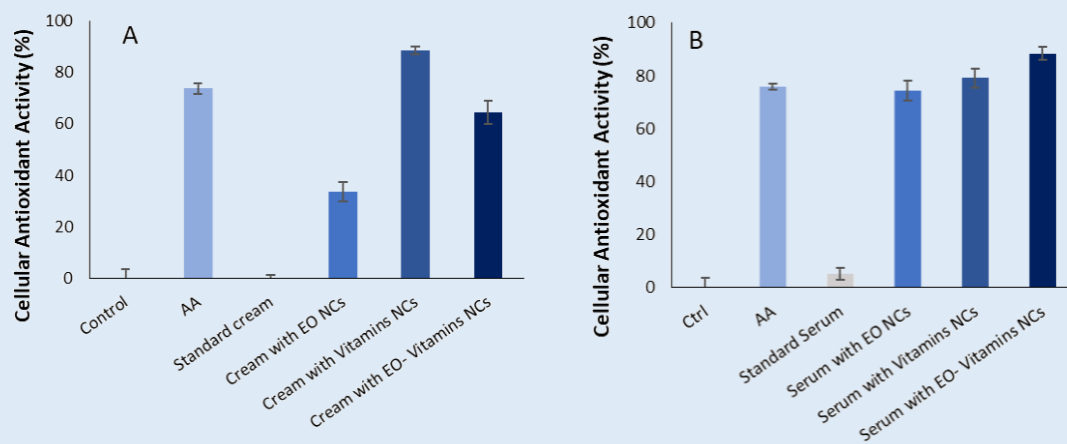


Figure 2. Antioxidant activity of 3% Cream (A) and 3% Serum (B) containing essential oil (EO) NCS, Vitamins NCS and EO-Vitamins NCS on skin fibroblasts. Ascorbic acid (AA) was used as an antioxidant control.

¹ Wolfe, K.L. and R.H. Liu, *Cellular Antioxidant Activity (CAA) Assay for Assessing Antioxidants, Foods, and Dietary Supplements*. Journal of Agricultural and Food Chemistry, 2007. 55(22): p. 8896-8907.

The cosmetic formulations were also submitted to an anti-elastase test, that proved their ability to inhibit the skin elastase, the enzyme responsible for breaking down elastin, the elastic fibre that together with collagen determines the mechanical properties of skin. It has been demonstrated that elastase plays a decisive role in wrinkles formation through the degeneration of skin elastic fibres.

In this assay, the anti-elastase efficacy of the cosmetic serum increased upon the incorporation of the essential oil nanocapsules and Vitamins nanocapsules (Figure 3). This effect is explained by the potential of the encapsulated actives to inhibit the specific enzymes, such as elastase.

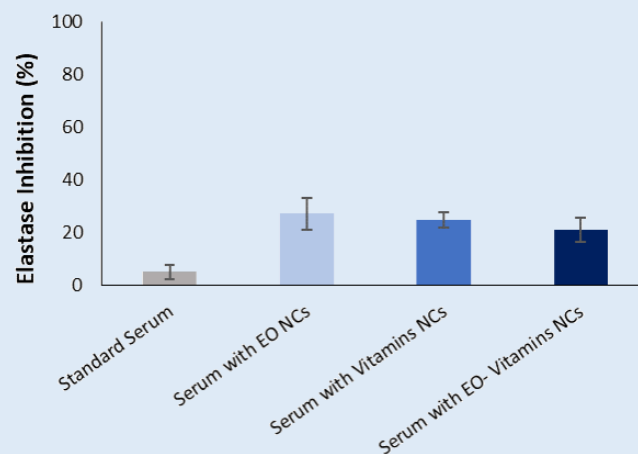


Figure 3. Anti-elastase activity of 3% Serum containing essential oil (EO) NCs, Vitamins NCs and EO-Vitamins NCs. All experiments were carried out in triplicate.

The cosmetic formulations developed (anti-wrinkle cream and anti-wrinkle serum) were submitted to several tests in order to assess their stability and safety regarding skin application, before they could be submitted to a use test/efficacy test (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 tests performed to the anti-wrinkle cream and serum to assess their stability and safety regarding skin application.

TEST	Anti-wrinkle serum	Anti-wrinkle cream
Accelerate ageing stability study	OK	OK
Microbiological control according 1223/2009	OK	OK
Patch test (Skin irritation)	OK	OK
Challenge (preservative efficacy)	OK	OK
Moisturizing effect	T4h:131%	T4h:151%
Irritation eye	OK	OK
Cellular antioxidant activity (CAA)	88,32%	68,94%
Transdermal Epidermal Water Loss (TEWL)	OK	OK

The cosmetic demonstrators were also evaluated by *in-vivo* efficacy studies. These studies were conducted by the application of the cream/serum in the face of 25 healthy subjects (women, ages between 25 – 50 years old, all types of skin) that have used the products for 28 consecutive days (Table 2 and Figure 4). All the tests were performed under dermatological control and a signed consent agreement.

Table 2. Efficacy tests to confirm the main anti-wrinkle properties of the demonstrators.

TEST	Anti-wrinkle serum	Anti-wrinkle cream
Product reduces wrinkles depth	17%	10%
Product reduces wrinkles width	10%	3%
Product reduces wrinkles length	4%	6%
Product reduces wrinkles count	16%	19%
Product reduces wrinkles volume	17%	7%
Product reduces wrinkles area	7%	8%
Product leaves feeling of skin moisturizing	96%	100%
Product shows off a radiant and young skin	92%	100%
Product helps to fight the first signs of skin ageing	79%	76%
Product reduces expression lines	71%	72%
Product has anti-wrinkle properties	75%	68%
Product has firming effect	83%	92%
Product rejuvenates skin	83%	92%
Product visibly reduces wrinkles	67%	64%
Product prevents the formation of new lines and wrinkles	71%	68%
Product provides comfortable skin care	96%	100%

After the 28 days of regular application of both the cream and serum, under the specific test conditions, it was concluded that the developed anti-wrinkle demonstrators:

- ✓ were tested under dermatological control.
- ✓ were very well tolerated at the application site.
- ✓ properties declared by the Customer have been confirmed.

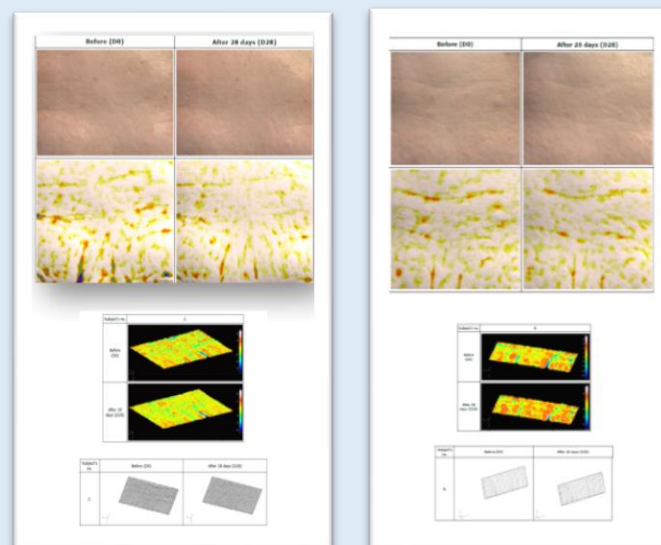


Figure 4. Pictures of face and neck before and after product application: right - anti-wrinkle serum; left - anti-wrinkle cream.

PRODUCT SPECIFICATIONS

The developed anti-wrinkle demonstrators have similar specifications, being the main difference between them the colour and the texture of the formulations.

Name: Anti-wrinkle serum

PHYSICO-CHEMICAL PROPERTIES:

- ✓ Colour, Odour, Appearance: Bright yellow/orange opaque emulsion. Odour due to its fragrance
- ✓ Viscosity (22 °C): 250.000-500.000 cps
- ✓ pH (22 °C): 5,0 – 6,0

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:

- ✓ Moisturizing emulsion of easy application and rapid absorption
- ✓ Contributes to a smooth and hydrated skin
- ✓ Non-greasy
- ✓ Reduce expression lines and have firming effect
- ✓ Help to fight the first signs of skin ageing
- ✓ Prevent the formation of new lines and wrinkles

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 all skin types

WARNINGS

- ✓ For external use only

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.

Currently, the market has several anti-wrinkle cosmetic ingredients available for skin anti-ageing like milks, hormones, acids, Botox[®], egg membrane, among others. Discussion about effectiveness and aggressiveness to skin is one of the main topics in the subject population. The differences between them are related to the feedback of the customers (feeling of the skin after application and results), price and quality. Anti-wrinkle products are commonly used daily by women, expecting the best results after the single application. Adding to this point, global anti-ageing market is expected to raise in the next years.

At SKHINCAPS project, two anti-wrinkle products were developed, focused in women between 25-65 years old. These demonstrators are designed to be applied daily to face skin, for fighting against the signs of skin-ageing, 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 anti-wrinkle creams developed would be classified at Medium-High level (lower price than big & famous competitors like Caudalíe, Neutrogena, La Roche Posay; higher quality than Olay, ROC, Caudalíe, similar to La Roche Posay and Neutrogena.)

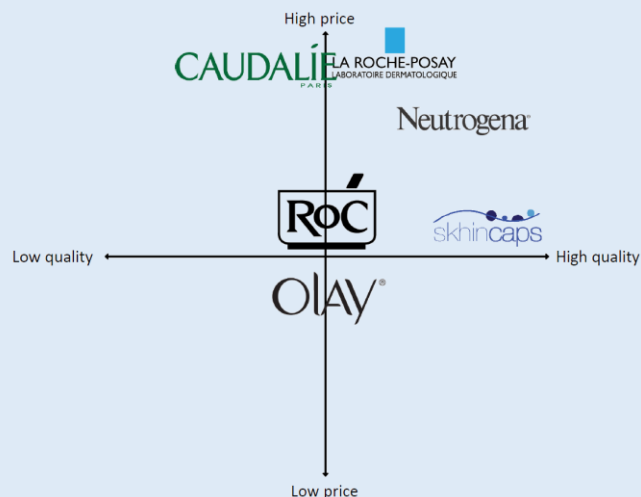


Figure 5. Positioning of anti-wrinkle demonstrators developed in SKHINCAPS project.

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.

SKIN ANTI-AGEING TEXTILES

The developed nanocapsules containing natural antioxidants were also incorporated on functional first layer textiles, aimed at improving skin anti-ageing.

Several trials were performed to assure the presence of the nanocapsules on the textile demonstrator and to evaluate their functionality. From Figure 6 it is possible to confirm the presence of anti-ageing nanocapsules onto cotton textile, comparing with control sample.

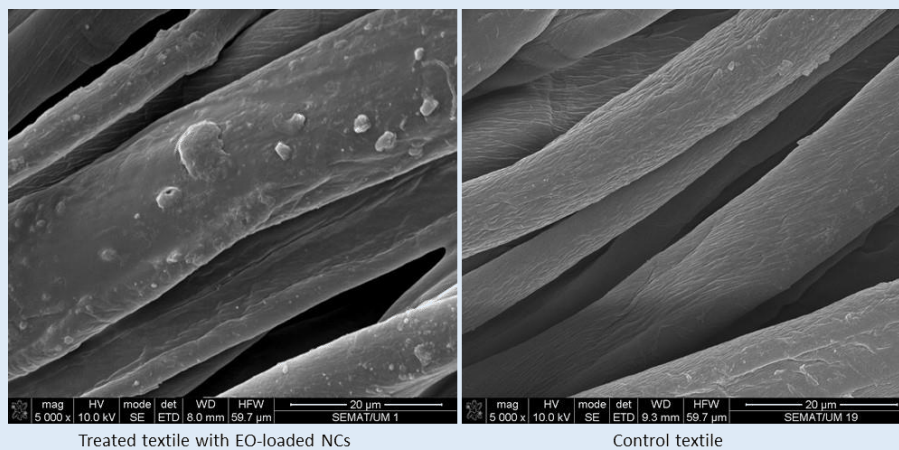


Figure 6. SEM micrographs (5 000 x) of the treated cotton textile with essential oil (EO) loaded NCs and untreated textile.

Franz cell studies with porcine skin were also conducted in order to better understand the performance of these textiles with anti-ageing nanocapsules in skin properties, and to evaluate the amount of active that penetrates skin over time (that is ultimately related with the textiles efficacy). The permeation profile was evaluated in the absence and presence of simulated sweat fluid, that was the trigger for the release of the actives from the nanocapsules.

Figure 7 shows the enhanced release of essential oil in the presence of sweat simulated fluid, confirming that the active release from these nanocapsules is triggered by the presence of salts. Thus, smart textiles with a controlled release can be developed based on these essential oil loaded nanocapsules.

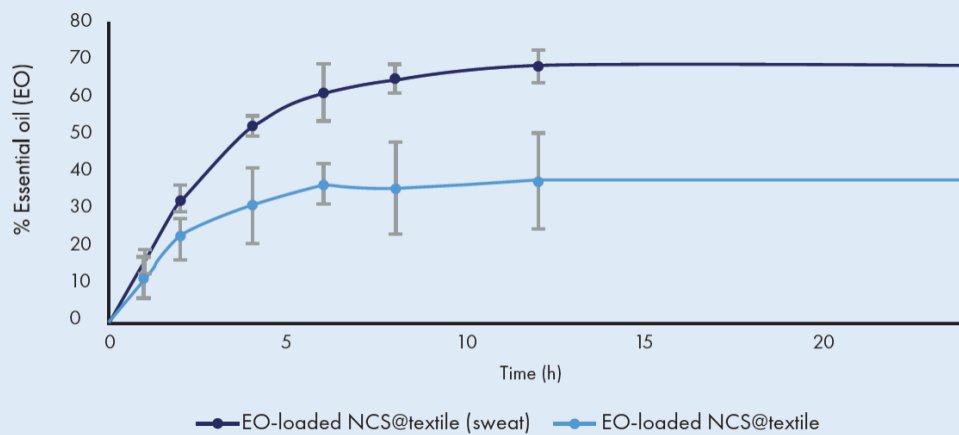


Figure 7. Percentage of essential oil (EO) in the receptor chamber at different time points, in the presence or absence of sweat (Franz cells methodology).

In order to evaluate the antioxidant property of the functionalized textile, a DPPH scavenging assay was performed. After 24 hours, the results show a higher antioxidant activity for the treated textile with essential oil loaded nanocapsules, indicating a good performance of this demonstrator (Figure 8).

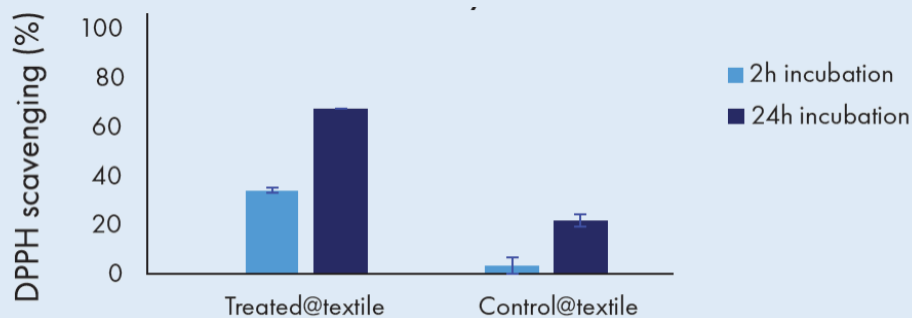


Figure 8. Antioxidant activity of the treated and control textiles (DPPH method).

Additionally, corneometry studies were performed to assess demonstrators' safety, by placing the developed textiles in contact with the skin of healthy volunteers for a period of 2 hours. It was verified that the textiles containing antioxidants loaded nanocapsules are harmless to the users, since no side effect was observed for the measured skin properties – erythema and transepidermal water loss.

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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).