Evaluation of the release of active ingredients from a bio-cellulose mask on support followed by a clinical evaluation of its efficiency in combination with a serum with a depigmentation activity

LATI Elian¹, DANIEL Magalie¹, DURAND Caroline¹, SANDAGER Elisabeth² and MONTASTIER Christiane²

1- Laboratoire BIO-EC, 1 chemin de Saulxier, 91 160 Longjumeau, France ; 2- Helena Rubinstein, 106 rue Danton, 92 697 Levallois-Perret, France

Keywords: Bioavailability, combination of cosmetic products, depigmentation, human skin explants, melanin, volunteers.

Introduction:

After proving the performance of a serum with depigmentation activity, the goal of this study is to demonstrate faster and more intense results, when using in combination a serum and a bio-cellulose mask on support, containing the same depigmentation active ingredients.

For this, we have shown the release of depigmentation active ingredients impregnated in a bio-cellulose mask on human living skin explants and we have evaluated the efficiency of a serum alone against this same serum in combination with the bio-cellulose mask, on a panel of 20 women volunteers.

Materials and Methods:

Ex vivo study and bioavailability

Preparation of human living skin explants

Human living skin explants were obtained from the abdomen of a healthy Caucasian woman (44 years) who had undergone aesthetic surgery. All subcutaneous fat was removed from the skin and 12 circular explants (~1 cm diameter) were excised using a sample punch. The explants were kept in survival in BEM culture medium (BIO-EC’s Explants Medium) at 37°C in a humid, 5%-CO₂ atmosphere [1].

Preparation of the tested product

The fluorescein isothiocyanate (FITC) product has been incorporated in the depigmentation active ingredients at the final concentration of 0.2%.

Application of the tested product

On day 0, the bio-cellulose mask was cut into 8 mm disc diameter. The tested product was deposited on the disc for one minute.
The bio-cellulose mask impregnated with the tested product was applied topically on 9 explants. After 10 minutes of contact, the discs were removed. The 3 explants from the control batch did not receive any treatment.

**Sampling**

On day 0, the 3 explants from the control batch were collected and frozen at -80°C. After 6h, 12h and 24h of survival, 3 explants from the treated batch were collected and frozen at -80°C.

**Histological processing**

The frozen samples were cut into 7µm-thick sections using a Leica CM 3050 cryostat. Sections were then mounted on Superfrost® plus silanized glass slides. The microscopic observations were realized using a Leica DMLB epifluorescence microscope. Pictures were digitized with a numeric DP72 Olympus camera with CellID storing software.

**In vivo study**

**The volunteers**

20 voluntary Korean women (35-50 years) with dull skin and hyperpigmented spots on their face were recruited. The volunteers agreed not to use face products during the period of the study, other than those provided. Moreover, they were not permitted to apply beauty products the evening before. They were informed of the possible adverse effects from using the products and the technical conditions in which the test was performed. They freely signed the consent form, which was written in compliance with the Declaration of Helsinki and the December 20th, 1998 act of the Code de la Santé Publique.

**Instructions for use the products**

The moist bio-cellulose mask and the serum were distributed to volunteers on day 0. They applied the serum on their whole face twice a day for 6 weeks. The bio-cellulose mask was applied once a week on only one half of their face for 5 weeks. The amount of serum was applied according to the judgment of the volunteers, depending on their cosmetic habits.

**Methods**

The efficiency of the serum used alone or the serum used in combination with the bio-cellulose mask was evaluated using 3 complementary techniques:

- The SIAscope, which allows using a non-invasive method to quickly measure the level of melanin in the skin.

- The Fontana-Masson method, which visualizes the melanin using tape stripping taken off from the skin surface [2].
- The clinical scoring method to evaluate the performance according to several criteria.

On day 0 and after 6 weeks, the technician in charge of the study stabilized the volunteers in a controlled-atmosphere room for 10 minutes and performed biometrological measures (SIAscope, D-squam swabs, clinical scoring).

**Results / Discussion:**

*Ex vivo study and bioavailability*

The quantification of fluorescein fluorescence on human skin explants, after microscopic observations conducted at 0h, 6h, 12h and 24h, shows a proper release of fluorescein fluorescence throughout all the different skin layers: the stratum corneum, epidermis and papillary dermis (*Table 1 and figure 1*).

![](image)

<table>
<thead>
<tr>
<th></th>
<th>0h</th>
<th>6h</th>
<th>12h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>standard deviation</td>
<td>Average</td>
<td>standard deviation</td>
</tr>
<tr>
<td>Stratum corneum</td>
<td>13,7 3</td>
<td>204,3 13,1</td>
<td>171,8 20,5</td>
<td>174,3 23,5</td>
</tr>
<tr>
<td>Epidermis</td>
<td>10,1 0,4</td>
<td>75,9 28,2</td>
<td>57,1 23,7</td>
<td>66,2 29,9</td>
</tr>
<tr>
<td>Papillary dermis</td>
<td>18,1 1,9</td>
<td>35,6 8,4</td>
<td>31,8 15</td>
<td>35,4 5,1</td>
</tr>
</tbody>
</table>

**Table 1 and Figure 1: Demonstration of the release of fluorescein isothiocyanate over skin explants**
At 0h, the fluorescein fluorescence was not observed on the stratum corneum surface neither in stratum corneum (SC) and in epidermis (Ep). A very low autofluorescence was observed in papillary dermis (PD), especially on elastic fibers (figure 2A).

6 hours after application of the bio-cellulose mask, the fluorescence was very strong on the SC surface, very clear in the SC, moderate to quite clear in Ep and weak in PD (figure 2B).

12 hours and 24 hours after application of the bio-cellulose mask, the fluorescence was comparable to that observed after 6 hours (figure 2C and 2D).

![Fluorescein fluorescence on human skin explants after microscopic observations conducted at 0h, 6h, 12h and 24h](image)
**In vivo study**

The different measurements performed with the SIAscope show a significant reduction of the level of melanin by 3% when using the serum alone for 6 weeks. This level of reduction reached 6% when the serum is used in combination with the bio-cellulose mask for 5 weeks (figure 3).

![Image of SIAscope measurements](image)

Figure 3: Example of images obtained with the SIAscope before and after the application of serum for 6 weeks or serum and bio-cellulose mask for 5 weeks.
The outcome of the Fontana-Masson method confirms the results measured with the SIAscope. Indeed, after 6 weeks of using the serum alone, 35% of the volunteers showed a reduction in melanin intensity within the corneocytes, while the consecutive use of the serum and the bio-cellulose mask in combination for 5 weeks induced a reduction of the melanin intensity in 47% of the volunteers (figure 4).

![Figure 4: Example of images obtained with the Fontana-Masson method before and after the application of serum for 6 weeks or serum and bio-cellulose mask for 5 weeks](image)

Furthermore, the results of the clinical scoring show that applying the bio-cellulose mask on top of the serum improves the brightness and radiance and homogeneity of the complexion. Skin is also smoother after the application of the bio-cellulose mask.
**Conclusion:**

As a conclusion, this whole study shows the release of fluorescein from the bio-cellulose mask, from 6 hours after the application of the tested product, to the different layers of the skin; the epidermis representing 50% of the impregnation of the stratum corneum.

Moreover, the clinical evaluation shows the serum alone or the serum in combination with the bio-cellulose mask significantly reduces the melanin level after only 5-6 weeks of use. Also, the study demonstrates that the use of the bio-cellulose mask on support reinforces the depigmentation activity of the serum.

**References:**
