The measurements of hydration degree after application of semisolid gels with peptide*

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Keywords: peptides; semisolidsgels; hydration degree

Abstract: In recent years, there has been an increased amount of scientific interest in the fields of cosmetology and pharmacology. Among other benefits, these disciplines explore ingredients that provide skin with a beautiful, healthy appearance, protect it from external factors and decelerate the ageing process.
The cosmetic industry has been developing towards research of active substances. A number of leading cosmetic lines have been enriched with new active ingredients, including synthetic peptides. The most popular peptides on the cosmetic market are signal peptides, also known as stimulating peptides.
In this experiment the measurements of skin hydration before and after the application of hydrogels with peptide were carried out on biological material derived from domestic swine. Tests were performed using special apparatus for skin testing. The results obtained refer to the degree of moisturising properties of the peptide depending on its sequence.

1. Introduction

The technology of cosmetic peptides has been developed by synthesis of new fragments of peptides, which imitate sequences occurring in proteins α-collagen and elastin. Peptides are used in cosmetic products as small molecules consisting of 6-7 amino acids, but there are also exceptions in the form of 8-10 amino acids, and sometimes even 20 amino acids. The sequence of amino acid has a direct impact on the size and nature of peptides (Lupo and Cole, 2007). Moreover, it can be observed which part of the molecule has been responsible for its biological activity. This makes it possible to create smaller and cheaper compounds with the same or similar effect (Griffiths, 2010).

*This work is financed from European Social Fund and supported by Grant BSRMNiUSD no. 538-8454-B013-13, DS/530–8454–D194–12–1E and DS/530–8210–D181–12–1F University of Gdańsk.

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The main task of moisturising cosmetics, including the hydrogel masks investigated, is to improve the degree of hydration of the skin, as well as its appearance (Łubkowska 2013). Specific peptides were designed with the purpose of improving the effects of cosmetics on skin. When peptides get to the appropriate place in the skin, they can improve its appearance. Besides, peptides with a corresponding sequence added to the formula of hydrogel mask can reduce the amount of preservatives needed in the general procedure. Normally, peptides are used in many skin care products to improve the force of moisturising cosmetics (Lupo, 2005).

Moisturising cosmetics, such as hydrogel masks are very popular skincare products. They have a very simple composition, are easy to use and are not expensive. In this experiment the formula of cosmetic product in question was improved by inclusion of a peptide which was synthesized in the Polypeptides Laboratory. Peptides as active ingredients in cosmetics showed the ability to transport ions, decrease facial muscle contraction and stimulate human skin fibroblasts. We attempted the synthesis of signal peptides because of the effects they have on the skin surface. A series of skin hydration measurements using a model of pig skin were made. In the end a number of results of the experiment confirmed beneficial effects of the additional peptide ingredient in hydrogel masks. Peptides, due to their appropriate sequence and in an adequate concentration, could induce significant effects of moisturising on skin.

2. Materials & methods

2.1. Peptides

The peptides Ala-Pro-Gly-Hyp andAla-Gly-Pro-Hypwere synthesized manually on the 0.1 millimole scale by a solid-phase method using the Fmoc/B^{tu}procedure according to a two-step procedure:

1. 5 and 15 min deprotection steps using 20% piperidine in a (1:1 v/v) mixture of DMF and NMP (in the presence of 1% Triton);

2. coupling reactions performed with the protected amino acid diluted in a mixture of a DMF and NMP (1:1 v/v) in the presence of 1% Triton and using 3 molar excess of DIC as the coupling reagent in the presence of HOBt. The reaction was carried out for 2 hours.

The completeness of each coupling reaction was monitored by means of the chloranil test (Atherton et al., 1987). If the test was positive, the coupling reaction was repeated using TBTU and HOBt in the presence of DIPEA, mixing for 2 hours.

The protected peptidyl resins were treated with a mixture of 95% trifluoroacetic acid (TFA), 2.5% water, and 2.5% TIS for 2 hours. After the cleavage, the solid support was removed by filtration and the filtrate was concentrated under reduced pressure. The cleaved peptide was precipitated with diethyl ether and lyophilized.

2.2. Semisolids gels

To prepare the gels the following ingredients were used: Aqua, Carbomer, Glycerine, Phenonip, D-Panthenol, Dimethicone and Triethanolamine. At the end 0.05% wt. peptide was added to the gel. The gels were prepared by weighing all ingredients, slowly adding water and shaking gently (to avoid the formation of bubbles) until

gel was formed. It was mixed on magnetic stirrer Eurostar Digital (*IKA Mischen*). In the end 50 mg of each gel formulation were received, which were stored at 4°C in the fridge.

2.3. Model skin and ethical aspects

The skin used in this experiment came from a domestic pig (*e mortuo*). In the opinion of the Bioethical Commission of the Medical University of Gdańsk, the study did not require any special authorization.

Model skin for cosmetic tests was prepared shortly before the testing started. The pig skin was taken off the sides of the animal.On its surface there were dead skin cells-corneocytes, which, like in the human epidermal appear in *stratum corneum*.

2.4. Measurements of skin hydration

The degree of the skin hydration was measured with cosmetic camera (BIOtronic). The camera incorporates the latest skin conductance technology. The water content of the skin model was defined on the basis of luminous diodes scaled in relative terms from 1 to 20 mS. The degree of hydration was examined before and after application of preparations on the prepared and disinfected pieces of porcine skin. The degree of hydration was measured in the following intervals: 10, 15, 30 and 60 minutes. All measurements were performed in neutral conditions of humidity and temperature in the laboratory.

2.5. Statistics measurements

With the development of experimental methods descriptive statistics were used (Korcz et al., 2008). The applied measurement method was sufficiently precise and sensitive. The arithmetic mean of the experimental results was calculated:

$$\overline{x}= \frac{x_1+x_2+\cdots+x_i}{n} \tag{1}$$

where: x_i - the value of the i-th measurement, and n - number of all measurements. The standard deviation (dispersion) scheduled according was calculated by the formula:

$$\sigma=\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i-\overline{x})^2} \tag{2}$$

The results are statistically significant. For a statistical analysis MS Excel was used (Ufnalski and Mądry, 2000).

3. Results

The peptides were characterized by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI–TOF-MS) and had the expected molecular weights (Bakry et al., 2007). The most intensive signals in the mass spectra corresponded to alkali metal adduct $[M+Na]^+$of analysed peptides. MALDI-TOF measurements confirmed the expected structure of synthesized products. The data is summarized in Tab. 1.

Other spectra were done by the technique of Thin Layer Chromatography (TLC), where R_f of obtained compounds was calculated, that is: the distance travelled by separated substance to the distance travelled by the front of eluent (developing structure) (Hancock and Battersby, 1976). The characteristics shown in Tab. 1.

Tab. 1. Physical-chemical properties of peptides

Sequences of peptide	Ion mass		Peptide mass [mg]	Productivity [%]	R_f
	Calculated	Designated			
Ala-Pro-Gly-Hyp	356	379.1 $[M+23]^+$	110	78	0.69
Ala-Gly-Pro-Hyp	356	379.1 $[M+23]^+$	120	87	0.73

Tests of the degree of hydration were performed for each hydrogel mask on the prepared biological material from pig. Measurements were performed for the reference hydrogel five times on different pieces of the epidermis.

The results are presented in Tab. 2 and Fig. 1 and Fig. 1. Graphs represent the measurements taken for the hydrogel mask with peptide in relation to the referential hydrogel. The first bar chart is presented for the average values of measurements taken for reference mask. The second bar chart shows the mean values of the measurements obtained respectively for: the hydrogel mask with Ala-Pro-Gly-Hyp and hydrogel mask with Ala-Gly-Pro-Hyp.

Tab. 2. The measurements of skin hydration before and after application of hydrogels

Hydration degree [a.u.]					
Time	before	after 10 min	after 15 min	after 30 min	after 60 min
Reference gel	10 ± 0.58	15 ± 0.65	16 ± 0.55	15 ± 0.89	14 ± 1.14
Gel with Ala-Pro-Gly-Hyp	10 ± 0.55	19 ± 0.45	20 ± 0.89	20 ± 0.89	20 ± 0.63
Gel with Ala-Gly-Pro-Hyp	10 ± 0.89	14 ± 1.23	16 ± 0.58	15 ± 1.14	14 ± 1.52

4. Summary

This study was conducted in order to justify the inclusion of the new sequences of peptides as components of skin care products. As practising dermatologists show, use of cosmetic peptides increases every year (Draelos, 2007).

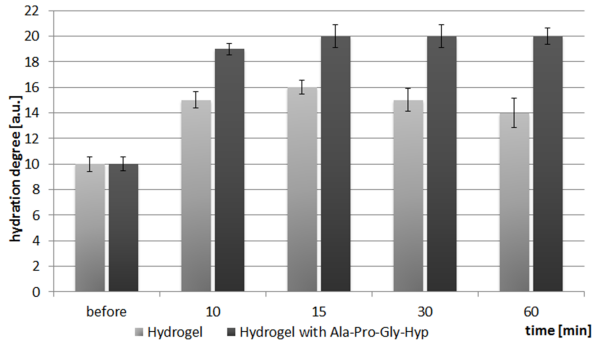

Fig. 1. The measurements for the reference gel and gel mask with Ala-Pro-Gly-Hyp

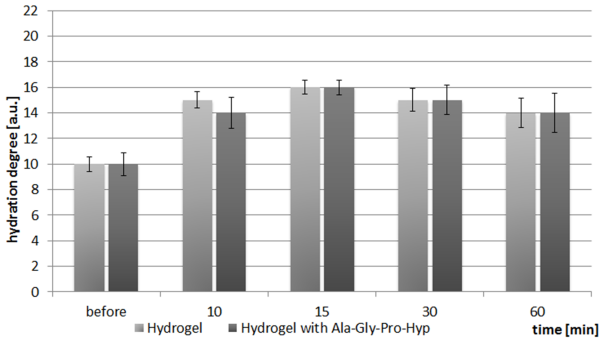

Fig. 2. The measurements for the reference gel and gel mask with Ala-Gly-Pro-Hyp

In the present study it should be recognized that the degree of hydration measurements were performed on pig skin which, although in terms of physiology is very similar to human skin, may not fully reflect its character. Currently, pig skin is the best equivalent of human skin and is used both in dermatological and medical research (Smorąg et al., 2011). During the trial faster absorption of hydrogel with peptides has been proven, as opposed to the hydrogel without the peptide used as reference. A colorless film on the surface of the skin model was noticed, which was absorbed over time. Hydrogel mask containing the Ala-Pro-Gly-Hyp showed longer moisturising effects on the model of skin which was due to the specific sequences of peptide.

With age, the degree of hydration of the skin decreases, skin becomes more dry, rough, loses water faster. The study has shown moisturising effects of hydrogel masks. Hydrogel masks with peptides helped keep moisture in the skin model for longer. Proper hydration of the skin is very important because it improves its appearance, gives softness, smoothness and firmness. The results are unequivocal proof that masks

with the proper sequence of peptide are able to improve skin hydration. It is believed that regular use can permanently improve the skin hydration.

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