Original Article

Physico-chemical and biological effectiveness evaluation of a liposome-enhanced mineral water on the skin

Avaliação físico-química e biológica da eficácia de uma água mineral lipossomada na pele

ABSTRACT

Introduction: Introduction: Mineral water has been used in Dermatology for its moisturizing and anti-inflammatory effects. Its penetration seems to be superficial. An elastic liposome containing mineral water was developed in efforts to improve its penetration and action in the skin.

Objectives: This study evaluated the efficacy of a 30-100 nm liposome, made of trehalose and soy phosphatidylcholine, which can cross 30 nm spaces and deliver the mineral water to deeper layers of the skin.

Methods: The RAMAN method was used to evaluate the permeation of the liposome into the skin. Its moisturizing effects were measured through the genic expression of filaggrin and acquaporin, and its anti-inflammatory effects were measured through NF-κβ quantities.

Results: After 30 minutes, the phosphatidylcholine was localized 1-7 nm deeper in the corneum layer; 60 minutes after, the liposome had penetrated to the spinous layer; and no changes were verified after 120 minutes. The genic expression of filaggrin increased 3 times and the expression of acquaporin increased 1.8 times. NF-κβ activity decreased 47%.

Conclusion: Nanotechnology and its benefits are very important to dermatology. The development of liposomes with Brazilian technology demonstrates the emergence of a knowledge base regarding cosmetic delivery systems in the country. Therefore, Brazil's wealth of mineral spring waters represents a considerably valuable resource and should be the object of further studies in dermatology. This study has verified that liposome-encapsulated water can permeate the skin and provide enhanced biological effects.

Keywords: mineral waters; liposomes; skin; Brazil; cosmetics.

RESUMO

Introdução: *Devido aos seus efeitos hidratante e anti-inflamatório, a água mineral tem sido progressivamente mais utilizada em Dermatologia. Não obstante, apresenta penetração aparentemente superficial. Um lipossoma elástico contendo água mineral foi desenvolvido para melhorar a sua penetração e ação na pele.*

Objetivos: *O presente estudo avaliou a eficácia de um lipossoma de 30-100nm, composto de trealose e fosfatidilcolina de soja, que pode atravessar espaços 30nm e carregar a água mineral para as camadas mais profundas da pele.*

Métodos: *O método de RAMAN foi utilizado para avaliar a penetração do lipossoma na pele. A análise dos seus efeitos hidratantes foi realizada através da medida da expressão gênica da filagrina e da acquaporina. Os efeitos anti-inflamatórios foram medidos através da quantidades de NF-κβ.*

Resultados: *A fosfatidilcolina foi utilizada como um marcador no teste de RAMAN, tendo sido localizada a 1-7nm mais profundamente no estrato córneo 30 minutos depois da aplicação; 60 minutos após a aplicação, o lipossoma foi encontrado sobre a camada espinhosa. Nenhuma alteração foi verificada 120 minutos após a a plicação. A expressão gênica da filagrina aumentou 3 vezes e a da aquaporina, 1.8 vez. Em relação à NF-κβ, houve 47% de diminuiçãoi na sua atividade.*

Conclusão*: A multidisciplinaridade da nanotecnologia e seus benefícios não podem ser minimizados. O desenvolvimento de lipossomas com tecnologia brasileira denotam a capacidade de nossos cientistas de inovar os sistemas de delivery em cosméticos, beneficiando a dermatologia. O aproveitamento da riqueza hídrica do Brasil também deve ser ressaltado a fim de estimular novos estudos relacionados com a nossa diversidade natural. O presente estudo permite concluir que a água lipossomada pode permear a pele e permite melhores efeitos biológicos.*

Palavras-chave: *águas minerais; lipossomos; pele; Brasil; cosméticos.*

Authors:
Samanta Nunes[1]
Bhertha Tamura[2]

[1] Dermatologist Medical Director, ZSN Associados – São Paulo (SP), Brazil
[2] PhD in Dermatology, Faculdade de Medicina da Universidade de São Paulo (USP) – São Paulo (SP), Brazil.

Correspondence:
Dra. Samanta Nunes
R. Diogo Moreira, 132/915 – Brooklin Novo
04569-011 – São Paulo – SP
E-mail: nunes.samanta@uol.com.br

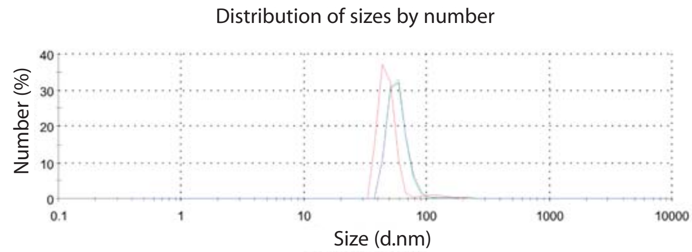
Received on: 28/11/2011
Approved on: 15/12/2011

This study was carried out at the Instituto de Bioengenharia da Pele – Evic Brasil Ltda. – São Paulo (SP), Brazil.

Financial support: Purainova laboratory (São Paulo – SP, Brazil) provided the material used in this study.
Conflicts of interest: Purainova laboratory (São Paulo – SP, Brazil) provided the material used in this study.

INTRODUCTION

Mineral waters are increasingly used in dermatology due to their physico-chemical properties, which make them beneficial in the treatment of various skin disorders. Their various effects (i.e., moisturizing, anti-inflammatory, calming, antiproliferative, antimicrobial, keratolytic, healing, and antioxidant) are the focus of interest in the therapeutic field. In order to improve the penetration of mineral water in the skin and the resulting moisturizing action, an elastic liposome that encapsulates molecules of mineral water from the Alvorada spring in the state of Sao Paulo, Brazil (liposome-enhanced mineral water) was developed.



Graph 1: Distribution of liposome particle sizes: three measurements (blue, red, and green)

Liposomes

Liposomes are spherical vesicles comprising one or more concentric lipid bilayers that isolate one or more internal aqueous compartments from the external environment. The high biocompatibility of liposomes, especially those from the natural lipids family, gives them a significant advantage over other drug delivery systems. Furthermore, liposomes are highly versatile systems; their size, lamellarity, surface, lipid composition, volume, and internal aqueous medium's composition can be manipulated according to the pharmaceutical and pharmacological requirements. The ability of these artificial vesicles to provide barriers against the diffusion of solutes was first demonstrated by Bangham in 1965.[1,2]

Traditionally, liposomes are obtained from a glycerophospholipid (phosphatidylcholine). More generally, they can be obtained from any lamellar phase-forming amphiphilic substance.[1,2] Since they are formed by amphiphilic compounds – meaning that their structure can encapsulate hydrophilic and lipophilic active substances – they have been widely used as a vehicle for dermocosmetic formulations. The main advantages of using liposomes for administering dermocosmetic agents are: their ability to transport hydro and liposoluble substances; their high affinity for biological membranes; the fact that they are made of natural biocompatible and biodegradable amphiphilics; and their potential to enhance the natural moisture of skin and hair. It is worth noting that such delivery systems can be quite different, and adaptations are made according to the liposomes' type and composition depending on the substance to be encapsulated. Liposomes are not stable in vehicles containing O/W or W/O emulsifiers. Even in the absence of surfactants, the incorporation of liposomes into certain vehicles can only occur under particular conditions, which can be induced by employing structural synthetic amphiphilics and standardizing the pH, osmolarity, and temperature.[3]

Liposome-enhanced Mineral Water

A unique elastic liposome, measuring 40-100 nm (Graph 1) and able to permeate skin pores of up to 30 nm, was developed to encapsulate a specific mineral water, to be applied on the skin using an aerosol product.

The mineral water encapsulated in liposomes was sourced from the Alvorada spring, located in the Serra do Japi, in Sao Paulo state's Atlantic forest region, in Brazil. Figure 1 shows a schematic drawing of the developed liposome. Figure 2 shows electron microscopy images of the liposomes dispersed in mineral water.

Previous studies have demonstrated *in vitro* biological effects of this mineral water, such as an increased genic expression of filaggrin and aquaporin 3 (important for hydration), a reduction of NF-kb activity (anti-inflammatory effect), and fibroblast proliferation. Further clinical studies are necessary to verify these effects *in vivo*.

Alvorada spring mineral water was encapsulated in specially developed liposomes to provide greater skin permeation of the potential biological active principle contained in it. The Alvorada source mineral water's lyophilized liposomes composition is shown in Table 1. Trehalose and soy phosphatidylcholine are the main components. Soy phosphatidylcholine represents about 90% of the lipids in the liposomes (Table 2).

OBJECTIVES

To evaluate liposomes' ability to enhance the skin permeation of Alvorada spring mineral water in vivo, using the Raman test. To evaluate the *in vitro* efficacy of liposome-enhanced water through the gene expression of aquaporin 3 and filaggrin and the nuclear transcription factor NF- 's activity level.

METHODS

Liposome-enhanced Mineral Water's Permeation of the Skin

Permeation tests using the Raman method were conducted in order to evaluate the efficacy of liposome-enhanced water. The tests used confocal microspectroscopy to determine the depth reached in the cutaneous tissue. The product was

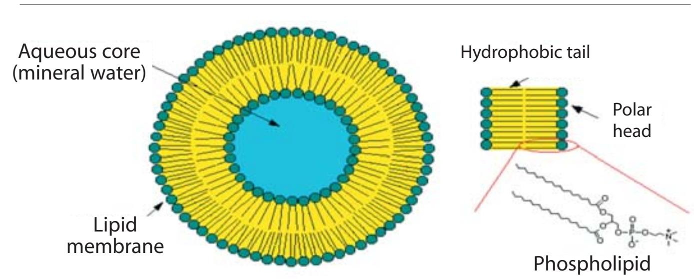


Figure 1: Schematic drawing of the liposome's configuration

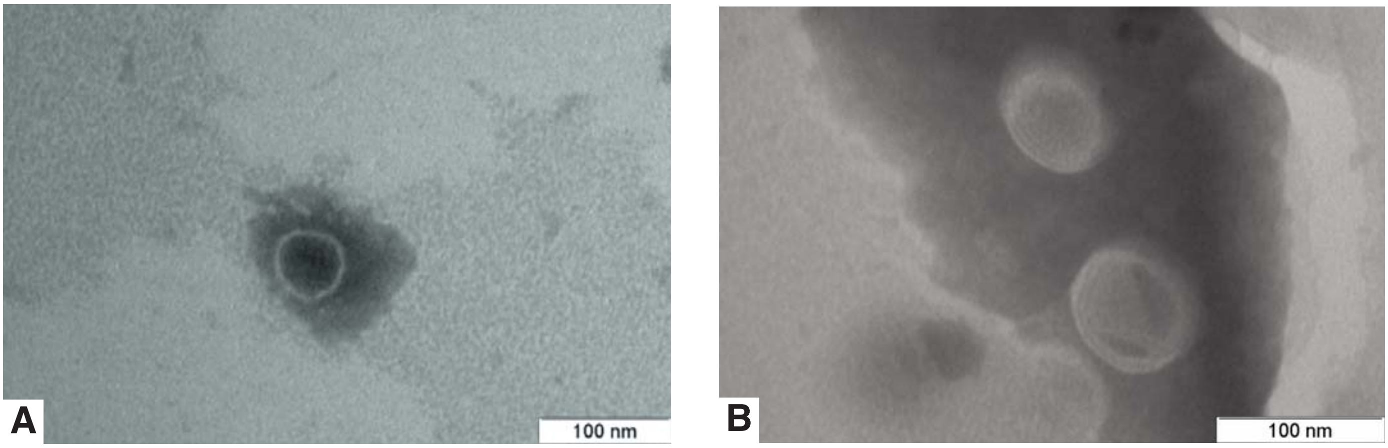


Figure 2: A and B. Transmission electron microscopy image of liposomes dispersed in mineral water

Table 1: Lyophilized (dry) liposomes' MP composition	
Raw material	**% (m/m)**
Trehalose	60
Soy phosphatidylcholine	35,9
Phytosterol ester	1,5
Cholesterol	1,2
Behenic acid	1,2
Ceramide 3	0,3

Table 2: Composição lipídica da MP lipossomas liofilizados	
Matéria-prima (lipídios)	**% (m/m)**
Fosfatidilcolina de soja	89,8
Éster de fitoesterol	3,8
Colesterol	2,9
Ácido behênico	2,9
Ceramidas 3	0,7

applied on the arms and measurements were taken at different time points (30, 60, and 120 minutes), using phosphatidylcholine as a marker of the liposome.

Stimulation of Filaggrin and Aquaporin 3 Gene Expression

Real-time Polymerase Chain Reaction (PCR) studies were performed to assess the gene expression of filaggrin and aquaporin 3. Human keratinocytes (Cascade Biologics, Grand Island, New York, USA) were cultured, and after six hours of contact with the mineral water, total RNA was extracted using TRI Reagent® Solution (Applied Biosystems, Carlsbad, California, USA) and quantified using the Quant-iT™ RNA Assay Kit (Invitrogen). Readings were carried out using the Quibit® Fluorometer device (Invitrogen, Grand Island, New York, USA) and the tests were conducted using a StepOnePlus (Applied Biosystems) device. The TaqMan® Gene Expression Assays trial system (Applied Biosystems) was used to measure the gene expression of filaggrin. The gene expression of aquaporin 3 was assessed with an EXPRESS One-Step SYBR® Green RNA-to-CT™ kit (Invitrogen). The relative amount of mRNA was calculated as described in studies by Pfaffl [4] and also Edith and Gregory. [5]

Decreased Activity of Nuclear Transcription Factor (NFκβ)

NF- activity was evaluated through the culture of human keratinocytes in six different concentrations of liposome-enhanced mineral water and demineralized water (control). The cells were subjected to UVA/UVB radiation and again exposed to the product for more than 24 hours. NF- 's activity level was measured using a kit manufactured by Cayman Chemical Company (Ann Arbor, Michigan, USA). The absorbance readings were taken using a microplate reader at 450 nm. NF- values were expressed as percentage ratios of the respective controls (basal and UVA/UVB).

RESULTS

Mineral Water Permeation of the Skin with Liposomes

After the first 30 minutes of the single application, phosphatidylcholine levels (liposome marker) were significantly increased at a 1-7 m depth (stratum corneum), suggesting that the liposome quickly permeated within the first minutes of application. In line with the observations of the first 30 minutes, reduced levels of phosphatidylcholine were found in the upper layers of the skin, and the levels progressively increased in deeper layers (with a peak of 19 m in the stratum spinosum), indicating that the liposome reaches deeper layers of the epidermis. Two hours after application, there were no significant alterations. No increase in phosphatidylcholine levels in the deeper layers was detected, and the permeation pattern remained unchanged from the level that was measured after the first 60 minutes. Results are shown in Graph 2.

Filaggrin Gene Expression Stimulation

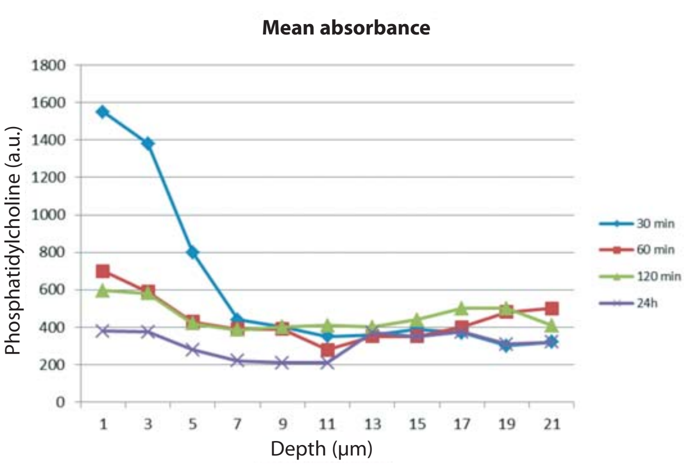
In a study of cell cultures, the liposome-enhanced mineral water provided approximately a threefold increase (2.8 times) in the gene expression of filaggrin – which was 7% higher than that obtained with the Alvorada spring water without liposomes. These data suggest that liposome-enhanced water can considerably contribute to the maintenance of the skin's hydric/balance, since filaggrin is a major protein forming the stratum corneum, and is responsible for the formation of approximately 50% of the skin's total natural moisturizing factor.

Stimulation of Aquaporin 3 Gene Expression

In a study of cell cultures, the Alvorada spring mineral water without liposomes produced a 1.8-fold increase in the aquaporin 3 gene expression compared to the Milli Q water, which did not contain minerals. In contrast, the test carried out with liposome-enhanced mineral water demonstrated a 4.3-fold increase (140% higher). This increase in the expression of aquaporin 3 confirms the product's ability to maintain the skin's hydric integrity, since that protein acts as a specialized mechanism capable of compensating excessive epidermal loss of water.

Decreased Activity of Nuclear Transcription Factor (NFκβ)

The 0.2% concentration of mineral water liposomes led to a significant reduction (47%) in NFκβ activity, suggesting a soothing and anti-inflammatory mechanism *in vitro*.



Graph 2: Phosphatidylcholine (liposome markers) measurements at baseline (0) and after 30, 60, and 90 minutes, and 24 hours after the single application of liposome-enhanced mineral water

DISCUSSION

One of the major disadvantages of topical therapy is the low speed of permeation of drugs applied to the skin. The barrier to the diffusion of most substances is the stratum corneum, which consists of corneocytes immersed in a lipid matrix. The stratum corneum's lipid matrix is formed of a lipid bilayer that is composed of cholesterol, fatty acids, and ceramides. The main advantages of using liposomes are linked to their ability to store water-soluble substances in their interior and lipophilic and amphiphilic substances in their membranes – in which the active substances are kept and transferred later to other membranes, such as the skin. As demonstrated by the Raman test, those liposomes are able to increase skin permeation, into the stratum corneum, and can better hydrate the skin. In addition, liposomes are non-toxic, biodegradable, and can be prepared in large scale.

In vitro tests also confirmed that the liposome-enhanced water presented advantageous biological effects compared with the mineral water without liposomes. Among these advantages, it is important to highlight the significant increase in the gene expression of aquaporin 3 and filaggrin, which denote an improved hydration and reconstruction capacity of the skin barrier. Many dermatoses can benefit from this new technology. A significantly greater reduction in the activity of nuclear transcription factor NFκβ was also observed, suggesting a greater anti-inflammatory mechanism compared to that of the unenhanced mineral water. These tests, combined with the *in vitro* tests conducted with the unenhanced water, suggest that the latter would be more appropriate in cases in which the cutaneous barrier is compromised. Liposome-enhanced water seems to be preferable for normal skin, where the liposomes increase skin permeation and deliver the water into the stratum spinosum.

Although it has not been proven clinically, the application of liposome-enhanced water on compromised skin could result in permeation that is too deep, which could cause irritant effects. Further studies are needed to better understand the effects of liposome-enhanced mineral water on healthy skin and dermatoses.

CONCLUSION

Nanotechnology's multidisciplinarity and benefits cannot be overlooked. The development of liposomes using Brazilian-based technology demonstrates that Brazilian scientists are innovating in the area of cosmetic delivery systems, which benefits the field of dermatology. Brazil's wealth of mineral spring waters represents a considerably valuable resource, and should be the object of further studies in dermatology. This study has verified that liposome-encapsulated water can permeate the skin and provide enhanced biological effects. ●

REFERENCES

1. Frézard F, Schettini DA, Rocha OGF, Demicheli C. Lipossomas: Propriedades físico-químicas e farmacológicas, aplicações na quimioterapia à base de antimônio. Quim Nova. 2005; 28(3): 511-518.
2. Lasic DD . Application of liposomes. In: Lipowsky, R., Sackmann, E, editors. Handbook of biological physics - Vol. 1B: Structure and dynamics of membranes – generic and specific interactions. Elsevier Science: The Netherlands, 1995. p. 491-520.
3. Chorilli M, Leonardi GR, Oliveira AG, Scarpa MV. Lipossomas em formulações dermocosméticas. Infarma. 2004; 16(7-8): 75-79.
4. Pfaffl MW. A new mathematical model for relative quantification in real time RT-PCR. Nucleic Acids Res. 2011; 29: 2000-7.
5. Gregory E. Miller, Edith Chen. Life stress and diminished expression of genes encoding glucocorticoid receptor and 2 adrenergic receptor in children with asthma. PNAS. 2006;103(4): 5496-501.