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라멜라-바이오 나노하이브리드: 3 Dimension-liposome을 이용한 카테킨(EGCG)에 안정화에 대한 연구

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Lamellar-bio nano-hybrid; The Study for Stability of Catechin (Green Tea : EGCG) Using 3-Dimensional Liposome

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요 약: 최근 고기능성 화장품이 출시 되면서 기능성 원료가 빛, 열, 산소에 매우 불안정하여 다양한 방법으로 안정성을 높이려고 연구되어 지고 있다. 특히, 카테킨은 주름 개선에 탁월한 원료이지만 빛, 열, 산소에 매우 불안정하다. 본 연구에서는 카테킨을 3 Dimension화 하여 안정성 및 피부 침투를 높였다. 1 dimension으로 sol-gel method로 실리카를 다공성으로 만들어서 다공성 부분에 카테킨을 흡착시킨다. 2 dimension으로 다공성 실리카에 흡착되어진 카테킨을 non-phospholipid 베지클을 이용하여 solid lipid nanoparticle (SLN)을 만든다. 마지막으로 3 dimension은 SLN되어진 카테킨을 skin lipid matrix를 이용하여 lameller phase self organization시킨다. 3 Dimension-카테킨은 일반적인 리포좀에 비해 빛과 열에 대한 color 안정성을 chromameter로 측정한 결과 5~10배 더 안정하였으며, HPLC 분석 결과 카테킨의 생존율이 3~5배 더 개선되었다. 또한 penetration effect를 측정한 결과 일반 리포좀보다 더 깊게 침투되었다. Wrinkle reduction effect를 한달 후에 측정한 결과 일반 리포좀보다 주름이 현저하게 감소되었다. 이러한 여러가지 실험을 위해서 Laser light scattering system, cryo-SEM, chroma meter, HPLC, image analyzer, microfludizer 등을 사용하였다.

Abstract: In these several years, as many people have been attracted by the functional cosmetics, there are a lot of study to enhance the stability of active ingredients for light, heat, oxygen, etc. in the academic and industrial field. Especially, catechin is well known as strong anti-oxidant, anti-inflammatory and reducing agent for oxidative stress but it is very unstable for light, heat, oxygen, etc. In this study, the stability and skin penetration of catechin are improved by 3-dimensional method. As 1-dimension, porous silica is prepared using sol-gel method, and then catechin is adsorbed in pores of silica. As 2-dimension, solid lipid nanoparticles (SLN) are obtained using non-phospholipid vesicles. Finally 3-dimension is completion through lamellar phase self-organization that combines SLN catechin with skin lipid matrix. We used laser light scattering system, cyro-SEM, chromameter, HPLC and image analyzer to analyze our 3-dimentional systems. According to chromameter date, the color stability of 3-dimensional catechin is enhanced by 5~10 times compared with general liposome systems. We also confirmed through HPLC analysis that 3-dimensional catechin is more long lasting. The effect of skin penetration and wrinkle reduction are improved, too.

Keywords: *catechin (green tea: EGCG), porous silica, SLN (solid lipid nano-particle), SLM (skin lipid matrix), 3-dimensional liposomes*

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1. Introduction

New concept of ingredients for the functional cosmetics is needed because of the trend of well-being and development of nano technology. Among them green tea has been used as an antidote for the first time in the imperial household of China in 3,400 B.C. The principal ingredients of green tea such as polyphenol, saponin, etc. are well known as having following effects, the inhibition of various kinds of edema, anti-aging, maintenance of healthy skin and so on. It prevents skin from roughening and gives skin elasticity and moisturizing effect for its leaves contains zinc, copper, iron, manganese, fluorine, caffeine, polyphenol, vitamin P and vitamin C that is included more than 5 times compared with lemon. Green tea also contains EGCG (epigallocatechingallate) that protects skin damage by ultraviolet lays and skin aging caused by the lack of lipid protein. E. Scholz *et al.* reported that dried leaves of green tea have 20% catechins and also contain epicatechin, epicatechingallate, epigallocatechin, EGCG, catechin and gallocatechin.

The content of polyphenol(-)-epigallocatechin-3-gallate called as EGCG is especially reported as 24~36 mg/g [Bronner W. (1998)], 73 mg/g [Lee M. (1992)], 23~51 mg/g [Schlesier K. (1999)] and 66 mg/g [Scholz E. (1998)]. EGCG has following efficacies/effects: anti-bacterial, anti-thrombotic, anti-viral, cholesterol lowing, anti-oxidant, anti-atherogenic, anti-inflammatory, anti-diabetic, anti-angiogenic, anti-arthrific, anti-cancer and increase of fat oxidation (Figure 1). In spite of having many advantages, it is not widely used. Because EGCG is very unstable for environmental factors such as light, heat, oxygen, etc.

Therefore in this study 3-dimentional liposome of EGCG is made to enhance the stability. Liposome [1, 2, 3] is spherical closed vesicles of phospholipid [phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), sphingomyelins, cardiolipids, plasmalogens, phosphatidic acid and cerebroside] bilayers and molecular groups in equilibrium with water phase at the same time.

The component parts of lipid have amphiphilicity in itself: hydrophilic (polar) property and lipophilic (non-polar) property from alkyl chains. So when liposome is dispersed in water phase, it forms bilayer entrapping the active ingredients in water phase. Liposome is

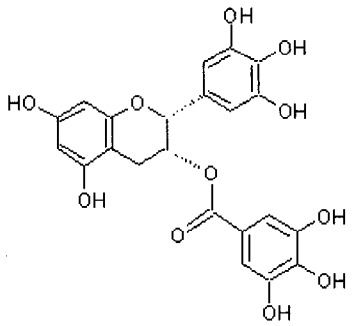

Figure 1. The structure of EGCG.

divided into two classes: MLV (multi-lamellar vesicles) and ULV (uni-lamellar vesicles) having LUV (large uni-lamellar vesicles) and SUV (small uni-lamellar vesicles). The diameters of MLV, LUV, and SUV are 400~3,500 nm, 100~1,000 nm and 20~50 nm and maximum contents of liposomes are 5~15%, 36~65% and 0.5~1.0% respectively.

Particle size analyzer, cryo-SEM, chromameter and HPLC are used to characterize 3-dimensional liposome compared with untreated an extract.

2. Materials and Methods

2.1. Equipment

We checked the color change of 3-dimensional liposomes using chromameter (CM-1000R, Minolta, Osaka, Japan) and measured the particle size and Zeta potential using laser light scattering system (Zetasizer 300 M, Malvern Ltd. UK). Microfluidizer (M 110 F, Microfluidics Corp. USA) and TK Auto Homomixer (Tokusbu, Kika, Kogyo, Japan) are employed for emulsification. Transmission electron microscope (JEM 1010, Jeol, Japan) and HPLC (LC-1100, HP, USA) are used to investigate particle and analyze EGCG content respectively. Steel (250×4.6 mm) as a column, hypersil-ODS 5 μm as a stationary phase and eluent A, acetonitril (99.9%), eluent B, 2% acetic acid (99.5%) in water and eluent C, methanol (99.9%) as a mobile phase have been used.

2.2. Materials

For this experiment, Lipoid S 100-3 (hydrogenated phosphatidylcholine) of Lipoid company and TEAVIGO (Roche) as EGCG are used. All other material used in our experiment are raw material for cosmetics. Finally,

Figure 2. FF-SEM image of 3-dimensional liposome.

we used purified water that had passed through an anion-cation exchange resin column.

2.3. Method

As the 1st dimension, porous silica is prepared by sol-gel method. And then, EGCG is incorporated in pores of silica by dispersion. For the 2nd dimension, SLN (solid lipid nano-particles) are obtained using stabilized EGCG by porous silica, cholesterol, cholesteryl ester, PEG-5-soyasterol, (POE)-n-cetylether, cetyl phosphate and cetyl palmitate. Lastly, 3rd dimension of EGCG is achieved using hydrogenated phosphatidylcholine (20%), propylene glycol (10%), glycerin (10%), ethanol (20%), caprylic/capric triglyceride (15%), water (25%) and secondarily stabilized EGCG by microfluidizer (1,000 bar, 3 passes).

3. Results and Discussion

3.1. Identification of MLV Formation of 3-Dimensional Liposome

As mentioned before, TEM and FF-SEM (freeze-fracture scaning electron microscopy) are used to identify the formation of secondary liposome and 3-dimensional liposome. As shown in Figure 2, it is seen that secondary liposome and SLM (skin lipid matrix) are well formed. In the case of 3-dimensional liposome, it has a shape of multi-lamella structure and secondary liposomes are located in 3-dimensional lamellar sheets and doubly capsulated by multi-lamellar sheets. Therefore we could observe that double stabilized EGCG is triply stabilized due to being situated in multi-lamella structure.

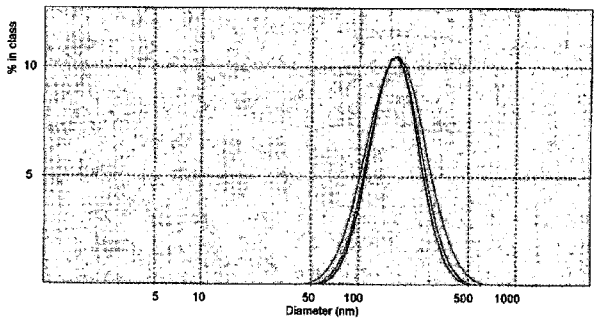

Figure 3. Particle size distribution of 3-dimensional liposome.

3.2. Particle Size Distribution of Secondary and 3-dimensional Liposome

We also studied for particle size distribution of secondary and 3-dimensional liposome with laser light scattering system, which is shown in Figure 3. Secondary liposome has the size distribution from 40 nm to 200 nm and mean diameter about 80 nm. Also we observed that 3-dimensional liposome is formed from 50 nm to 500 nm and mean diameter about 250 nm. We think the reason why size distribution of 3-dimensional liposome is bigger than secondary one is that multi-lamellar vesicles of SLM is contributed to that of secondary one.

3.3. Color Stability of General and 3-dimensional Liposome Against Heat and Light

General and 3-dimensional liposome are stored for one month under following conditions: 25℃+without light, 40℃ and light exposure. In one month, color change is measured by chromameter. As shown in the Figure 4, we observed that color of EGCG varied with heat and light as time goes by. Under light exposure condition, 3-dimensional liposome is more stable by about 10 times compared with general one. Under 40℃ and 25℃+without light condition, it is 5 times and 4 times, respectively. These results mean that 3-dimensional liposome is more stable than general one because it is triply stabilized by means of porous silica and multi-lamellar sheet of 3-dimensional structure.

3.4. Variation of EGCG Content

Variation of EGCG content for two month is shown in Figure 5. It was showed that 3-dimensional liposome was 4 times as stable as general one under

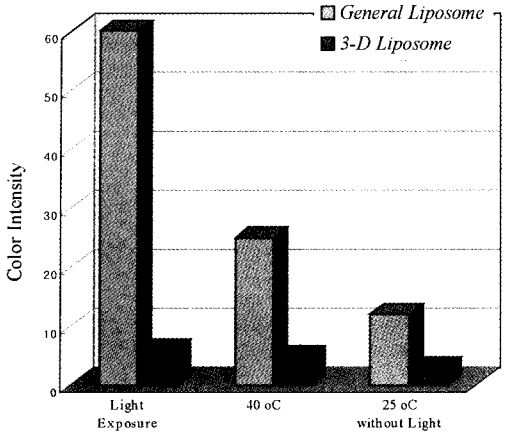

Figure 4. Color stability of general and 3-dimensional liposome against heat and light.

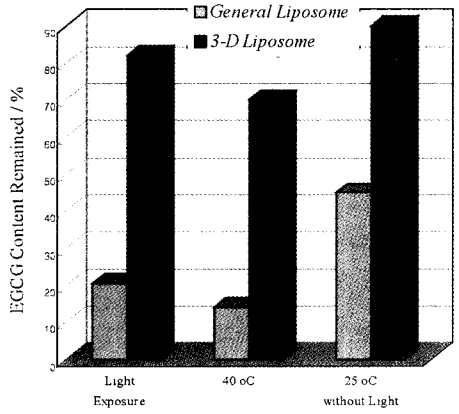

Figure 5. Variation of EGCG content.

light exposure, 5 times as stable at 40℃ and 2 times as stable at 25℃+without light. It is appeared that 3-dimensional liposome is more stabilized than general one due to triple stabilization.

3.5. Skin Penetration Effect of Liposomes

A 8-week-old hairless guienia pig (strain IAF/HA-hrbp) is used to study skin penetration effect. We put the abdominal skin of guienia pig cut off in Franz-type diffusion cells (Lab Fine Instruments). Receptor of cell (5 mL) is filled with 50 mM phosphate buffer (pH 7.4 and 0.1 M NaCl). Solution is mixed and dispersed with 600 rpm while the cell temperature is keeping at 32℃. Produced solution (200 μm) is poured in dornor container. As prearrangement timetable, solution is absorbed and diffused. At this time, skin area absorbed and diffused is 0.64 cm^2. After absorption and diffusion

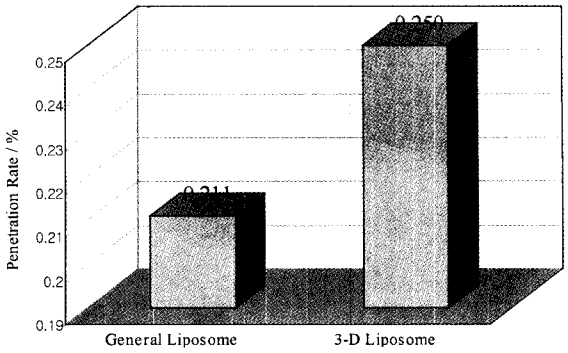

Figure 6. Skin penetration effect.

of active ingredient, the remainder on skin is washed off with wetted kim-wipes using 10 mL purified water. And then skin that active ingredient is absorbed and diffused is grinded with tip-type homogenizer (Polytron PT 2100, Switzerland). EGCG penetrated into skin is extracted using 4 mL ethanol. An extract (0.45 μm) is filtered by nylon filter membrane and EGCG content is measured by HPLC.

Two different types of cream containing 2 wt% of general and 3-dimensional liposome are prepared, respectively. After we applied the cream onto the skin of a hairless mouse, EGCG content is measured by HPLC. As shown in Figure 6, skin penetration of general liposome is about 0.211% and that of 3-dimensional one is about 0.250%. Its penetration efficiency is enhanced by 60%. In terms of this result, we could think 3-dimensional liposome improves skin penetration of EGCG because it is composed of phospholipid, cholesterol, ceramide 3, etc. that organize the skin structure materials.

4. Conclusion

In order to improve EGCG stability, we introduced 3-dimensional liposome system which is prepared by combining secondary liposome (SLN) and SLM. Secondary liposome is 40~200 nm in size (mean diameter ~80 nm). 3-dimensional one is 50~500 nm (mean diameter ~250 nm). 3-dimensional liposome system is more stable than general liposome: for color stability, 4 to 10 times, for livability of EGCG, 2 to 5 times. Skin penetration of 3-dimensional system is superior to that of general one by about 60%. The reason of enhanced stability and penetration is that it is triply stabilized by

porous silica, SLN and SLM organized the skin structure materials.

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