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5-Aminolevulinic Acid-Loaded Gel, Sponge Collagen to Enhance the Delivery Ability to Skin

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***Abstract*—**Topical photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) is an alternative therapy for treating superficial cancer, especially for skin or oral cancer. ALA, a precursor of the photosensitizer protoporphyrin IX (PpIX), is present as zwitterions and hydrophilic property which make the low permeability through the cell membrane. Collagen is a traditional carrier; its molecular composed various amino acids which bear positive charge and negative charge. In order to utilize the ion-pairs with ALA and collagen, the study employed various pH values adjusting the net charge. The aim of this study was to compare a series collagen form, including solution, gel and sponge to investigate the topical delivery behavior of ALA. The in vivo confocal laser scanning microscopy (CLSM) study demonstrated that PpIX generation ability was different pattern after apply for 6 h. Gel type could generate high PpIX, and archived more deep of skin depth.

***Keywords*—**5-Aminolevulinic acid (ALA), Collagen, Ion-pairs, Penetration behavior

I. Introduction

TOPICAL photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) is an alternative therapy for treating superficial cancer, especially for skin or oral cancers [1], [2]. PDT is a composite technique which requires three basic elements: a photosensitizer, light irradiation, and singlet oxygen. ALA, a second-generation photosensitizer, is a precursor which generated protoporphyrin IX (PpIX) after the exogenous application of ALA in vivo condition [3]. In addition, ALA molecules are zwitterions which carry both a positive charge at the amine terminal and a negative charge at the carboxylic terminal [4]. These characteristic compounds have limited capacities to reach and ultimately enter target cells within a biological environment. Overall, the major limitation of PDT is the poor penetration of ALA through biological barriers like cell membranes or the skin, due to its hydrophilic characteristic and charge [5], [6].

Recently, numerous strategies were proposed to improve these penetration problems. The notable of ALA preparations was Levlan Kerastick® in clinical usage of. However, the dosage form is solution type makes inconvenience usage, and the high concentration of 20% ALA for clinical usage increases skin irritation and costs [7], [8]. The aim of the study was to employ a solid or semi-solid carrier to carry ALA and efficiently delivery into skin. Collagen is a traditional carrier; its molecular composed various amino acids which bear positive charge and negative charge [9]. Moreover, collagen is well biomaterial in bioengineering field due to its natural properties and the low toxicity and the low immune response [10], [11].

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In the aspect of production and clinical usage, collagen was used in a various physical form such as solution, gel, sheet, sponge, powder. In order to utilize the ion-pairs with ALA and collagen, the study employed various pH values adjusting the net charge. The study was to compare a series collagen form, including solution, gel and sponge to investigate the topical delivery behavior of ALA in in vivo condition.

II. Experimental

A. Preparation of gel and sponge collagen

Liquid type collagen was purchased from Sunmax Biotechnology CO., LTD (Taiwan). The liquid type collagen and ALA was adding 10X PBS, then placed in oven at 40°C, and gel type collagen will be obtained. The sponge-type collagen was adding glutaraldehyde (0.05%) as cross-linking agent, and ALA was added. Next put in the -20°C and -80°C sequentially for 24 h. Finally, employed freeze-drying for 24 h, the sponge-type collagen will be obtained [12].

B. Determination of zeta potential

ALA and collagen prepared in different pH value zeta potential were measured by laser light scattering (LLS) with a helium–neon laser at 630 nm (ZS 90, Malvern, Worcestershire, U.K.) at 25°C.

C. In vivo topical delivery of ALA with different collagen type

Female nude mice (ICR-Foxnl) at 7–8 weeks old were used for the experiments. A glass cylinder with an available area of 0.785 cm^2 was placed on the dorsal skin of a mouse with glue. ALA of liquid, gel and sponge type was placed in cylinder, which was covered with parafilm. After being applied for 6 h, mice were sacrificed. Each skin site was wiped 10 times using a cotton-tipped stick with distilled water, and the treated area was excised. The amount of ALA induced PpIX retained in the skin was measured by CLSM. All procedures were carried out in the dark to prevent the influence of ambient light.

D. Confocal laser scanning microscopy (CLSM) study

For analysis of PpIX expression, CLSM was used to scan the fluorescence signal of PpIX at different skin depths. The excised nude mouse skin was positioned on the microscopic slide with the SC side face to the cover glass. Optical excitation was carried out with a 488 nm argon laser beam and fluorescence emission was detected at 590 nm. The PpIX intensity and the permeated depth were detected by a Leica TCS SP2 confocal microscope (Wetzlar, Germany) with Leica Confocal Software version 2.61. Each skin sample was sliced in sections of 40 μm thicknesses through the Z-axis by CLSM. Since CLSM is not able to be calibrated, we used arbitrary unit (A.U.) to compare the data.

E. Scanning electron microscopy (SEM)

The morphology and porosity of the sponge collagen were observed by Hitachi S5000 model scanning electron microscopy (SEM).

III. Results and Discussion

Numerous investigations have development potential carrier to improve penetration problem of ALA, such as emulsions, liposomes, ethsome, a lipid sponge form, and a nanocolloid lotion [13]–[16]. However, the carriers were liquid type make use inconvenience in the clinical. The present study demonstrates that topical delivery of ALA using gel and sponge type collagen as carriers which can successfully resolve the delivery problem. We established the penetration behavior of ALA-loaded gel and sponge collagen by using CLSM technique to observe the intensity of PpIX generation and penetration depth.

A. Ion-pair with ALA and collagen

ALA and collagen separate dissolved in the different pH value, the zeta potential was measured. The results demonstrated that collagen was neutralized when in the pH 6–7.4; when pH adjusted to 3–5, collagen presented positive charge; when pH adjusted to 8–10, collagen presented negative charge. On the other hand, ALA was represented net charge at pH 5.5 due to the amine (–) and the carboxylic (+) group, the result as shown in Figure 1.

Hence, the study was utilized ion-pair concept to combined ALA and collagen. ALA and collagen was dissolved in pH 7.4 and pH 3, separately. Collagen provides positive charge paired with negative charge of ALA. The charge binding capacity is the important concern, which relative about the release rate and kinetics. Hence, the following study employed in vivo study to understand the PpIX generation efficiency.

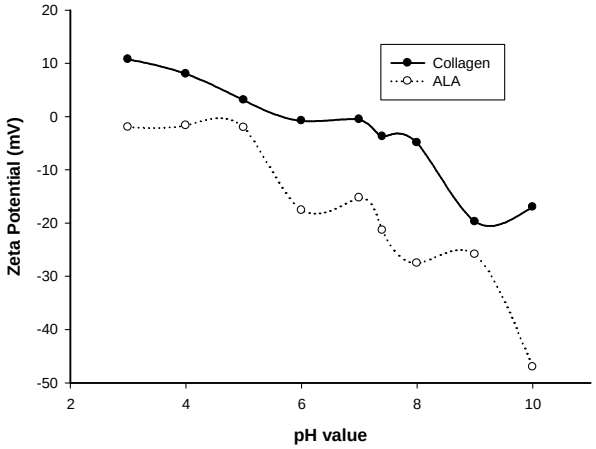

Fig. 1 The profile of different pH value v.s. zeta potential in ALA and collagen

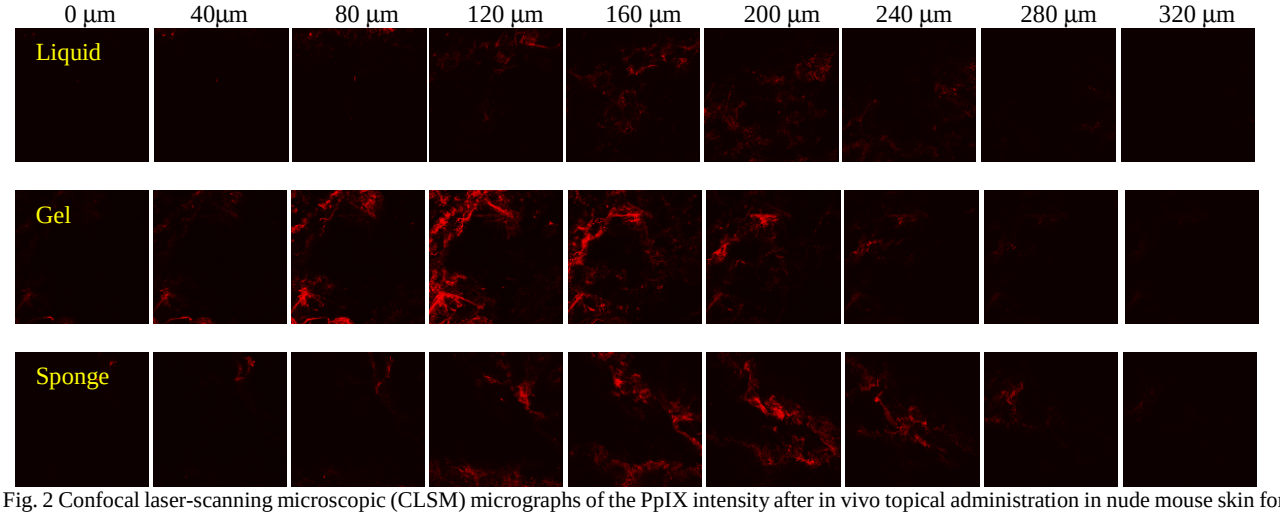

Fig. 2 Confocal laser-scanning microscopic (CLSM) micrographs of the PpIX intensity after in vivo topical administration in nude mouse skin for 6 hours. Images represent ALA-loaded liquid type collagen (commercial aqueous solution), gel type collagen, and sponge type collagen sequential scans in the xyz plane, and the full thickness was divided into nine segments from the surface of the skin (left to right)

B. Penetration behavior

In order to understand the penetration behavior of ALA-loaded liquid, gel and sponge type, the penetration depth and PpIX intensity was examined by using CLSM technique. To put it more concretely, CLSM images were quantitation of PpIX intensity. CLSM images data suggested the accumulation, distribution, and skin penetration depth of pTT, as shown in Figure 2, 3 and 4.

ALA-loaded liquid type collagen as negative control group which presented few PpIX accumulation, particular localized at upper epidermis about 200 μm. Both of ALA-loaded gel and sponge type collagen showed significantly increased PpIX intensity, and the penetration pattern were different. ALA-loaded gel type collagen showed a significantly higher intensity of PpIX than the liquid and sponge type collagen ($p < 0.05$). Moreover, ALA-loaded gel type collagen showed the higher PpIX intensity then liquid and sponge type collagen; and

the PpIX intensity were major located from 40-240 μm, particular at depth of 120μm. On the other hand, ALA-loaded sponge type collagen could convert PpIX and penetrated archived 120-240 mm, and the PpIX intensity higher than liquid type collagen.

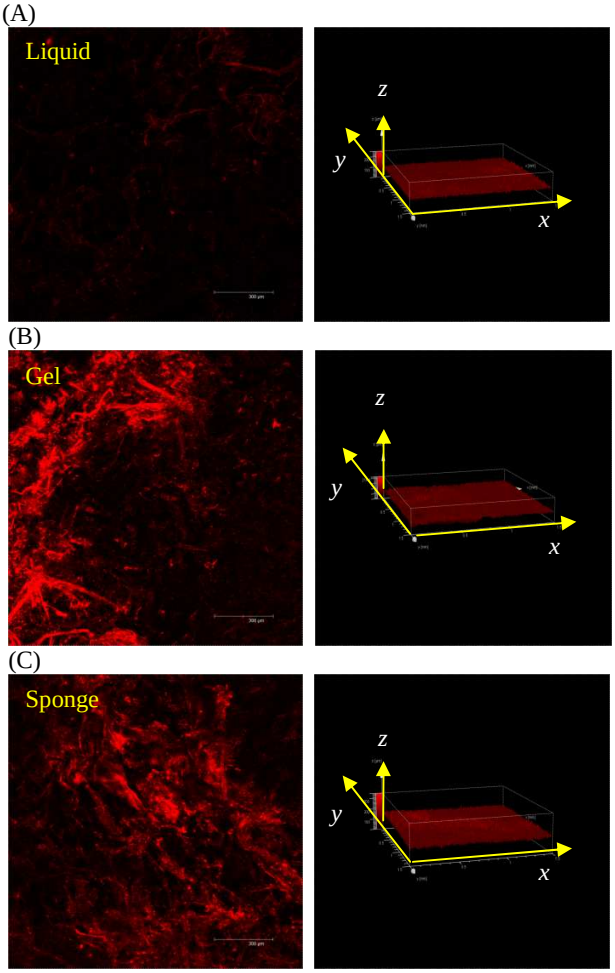

Fig. 3 Accumulative xyz images of all optical sections were merged from 0–320 μm in xyz scan. ALA-loaded (A) liquid (B) gel (C) sponge type of collagen

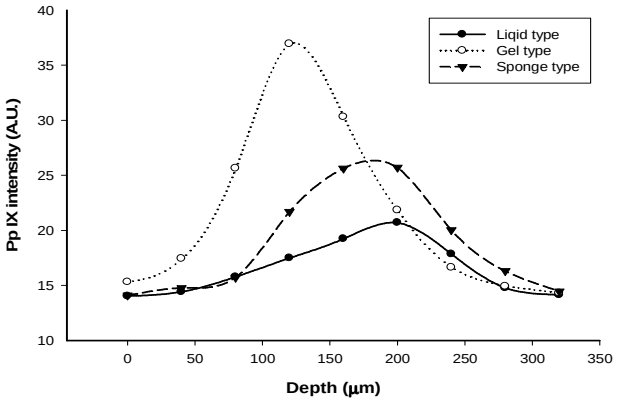

Fig. 4 Profile of skin depth versus intensity of liquid, gel and sponge type of collagen by quantification from CLSM micrographs

Indeed, ALA is a precursor of the photosensitizer, protoporphyrin IX (PpIX), formed in vivo after the exogenous application of ALA. After exogenous ALA topical applied to skin, PpIX is converted within mitochondria [17], [18]. The process may accumulate due to the limited capacity of ferrochelatase to convert it to heme. Overall, the results demonstrated that gel type collagen group has a broad distribution of PpIX intensity and the penetration depth extended from the stratum corneum to upper epidermis. In contrast, the sponge type collagen still presented enhancing effect, in spite of lower potential of penetration. However, sponge type collagen could release ALA and transform to PpIX, which accumulation archive more deep when compare with gel type collagen.

SEM images of the sponge collagen were shown in Figure 5. The results suggested that the matrix of sponge type collagen play a particular role in the cross linking matrix of physicochemical property. The construction of sponge collagen may explain the lower accumulation effect of PpIX. The phenomenon could be contributed by the thermodynamic effect. It implies that the amount of PpIX in the skin was significantly lower and might be confined to the release stage from the sponge system.

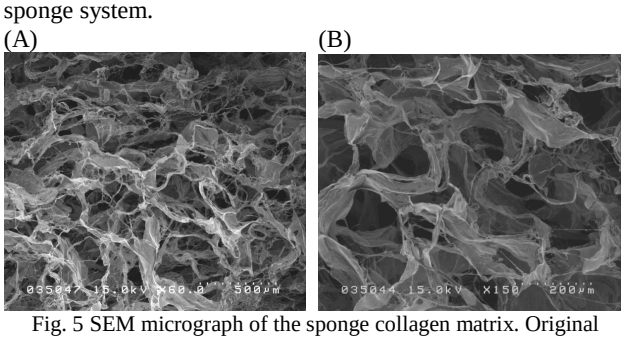

Fig. 5 SEM micrograph of the sponge collagen matrix. Original magnification (A) x 60 (B) x150

IV. Conclusion

In summary, gel and sponge type of collagen containing ALA was investigated in penetration behavior by CLSM technique. Results of CLSM indicated that the penetration ability of gel type collagen was greater than that of sponge type collagen in terms of PpIX accumulation in the skin, and the influence depth could reach about upper epidermis. It applies in the superficial lesion of disease. Moreover, the sponge type collagen was lower than that of gel type, which implies that PpIX is retarded in the skin possibly due to restrictions at the release stage from the matrix of sponge collagen.

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