

Transdermal delivery of hydrophilic dye by low-frequency ultrasound

低周波超音波による親水性染色色素の経皮投与

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1. Introduction

Transdermal drug delivery (TDD) is effective for therapy of skin disease. Additionally, this method can become an alternative to common delivery methods such as oral administration and injection. For recent decades, ultrasound technique is expected to be applied to TDD. In this technique, it is supposed that ultrasonic cavitation increases the permeability of skin, particularly that of stratum corneum which is outstanding barrier against the drug permeation.^[1] Therefore, low-frequency ultrasound, which can easily induce cavitation, is often used for TDD. Based on the histological and chemical analysis, the effect of ultrasound on the drug introduction through the skin have been intensively studied.^[1] However the detail introduction mechanism are still unknown because of few examination in terms of cavitation dynamics.

In this paper, we demonstrate the introduction effect induced by low-frequency ultrasound, using hydrophilic dye as the alternative of drug. Moreover, we discuss the effect of cavitation on the introduction, based on optical observations of cavitation behaviors using a high-speed video camera.

2. Materials and Methods

2.1 Material

Porcine skins on lateral flank and back (MAEDA Pork) were used. The frozen skin was unfrozen at room temperature and divided into samples with the area of about 15 mm square using scalpel. These skin samples included stratum corneum, epidermis, dermis and subcutaneous tissue. The thickness of the sample was from 3 to 5 mm. Paying special attention to excess damages of the skin surface, hairs was removed from the sample with the use of razor.

For the staining of the skin sample, sulforhodamine B (SRB) (Wako) was used as a hydrophilic colorimetric dye. We can clearly evaluate the introduction effects related to ultrasound using the hydrophilic colorimetric dye,

because it is difficult that hydrophilic molecule permeates the skin under normal condition.

2.2 Ultrasound treatment and staining protocol

Figure 1 shows a ultrasonic radiation system. Two Langevin transducers fixed to an acrylic chamber were faced each other. These transducer were driven by 10000 cycles sinusoidal signal with a center frequency of 27-28 kHz. The ultrasound radiation was repeated at interval of 5 sec (duty ratio, 0.067) for 30 minutes. During ultrasonic radiation, acoustic standing wave was formed in the chamber. A anti-node of sound pressure were located in the center of the chamber. The node lay on the edge of the chamber. A skin sample bound by two acrylic plates was set at the anti-node and the skin surface was located at the distance of about 9 mm from water surface.

Two staining protocol were conducted. One is the pre-treatment of the ultrasound before the staining. In this case, the skin sample was stained in aqueous solution of SRB for 30 min. The concentration of SRB was 0.5 % (w/v). The other is the staining simultaneously with the ultrasound treatment. The skin sample was sonicated in the chamber filled with 0.05 % (w/v) of SRB in water. In each protocol, control experiments, that is, only the staining of the skin sample were performed to examine clearly effects of ultrasound.

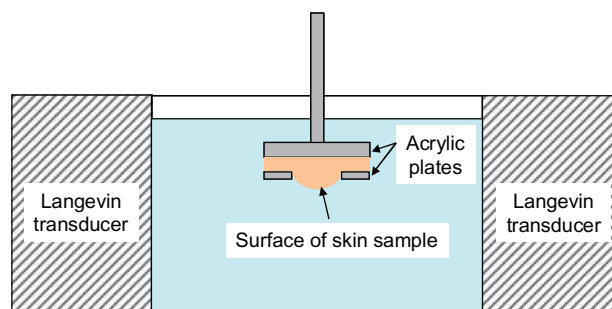


Fig. 1 Ultrasonic radiation system and fixation of skin sample.

2.3 Optical observation

To investigate effects of cavitation on the introduction of SRB, we observed cavitation behaviors on the surface of skin sample, using high-speed video camera (Shimadzu, HPV-1). The detailed observation system is described in Ref [2].

3. Results and discussions

3.1 Introduction effect

Figure 2 shows the results of the staining experiments using SRB. Images (a-1) and (a-2) were obtained in the staining after the ultrasound treatment. Images (c-1) and (c-2) were obtained in the staining simultaneously with the ultrasound treatment. Images (b) and (d) were control experiment of images (a) and (c), respectively.

In case of the skin samples without the ultrasound treatment [see images (b) and (d)], we could not confirm the significant permeation of SRB. On the other hand, we could clearly find the spotty gray regions [see images (a-1) and (c-1)]. This shows that SRB permeates the skin. Moreover, the permeations in the case of staining after ultrasound treatment indicate that irreversible alterations probably occur in the skin sample. These results agree with the previous studies.^[1] However, ultrasound treatment did not always result in the permeation of SRB [see images (a-2) and (b-2)]. The reproducibility of the permeation will be examined in detail.

3.2 Cavitation behaviors

Figure 3 shows the typical observed behaviors of cavitation generated by ultrasound. Radiation condition of ultrasound was same as the abovementioned conditions. Observed duration was 400 ms in which we could observe cavitation behaviors during 10000 cycles of the radiated ultrasound. Observed time is displayed on each image, where 0 sec is referred as the onset of ultrasound radiation.

In observed images (b) and (c), we can see that a hair is pushed toward the skin surface. Because this phenomenon did not occur without the generation of cavitation, it was supposed that ultrasonic cavitation induces the localized force pressing the skin surface for a duration of several hundred milliseconds. The observed permeation of SRB into the skin may be induced or enhanced by this force. However, we require further investigations on the mechanism of the force induced by cavitation and the relationship between cavitation and introduction effect.

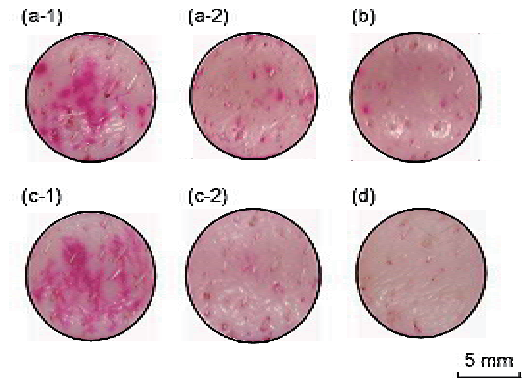


Fig. 2 Introduction effect of SRB into porcine skin caused by ultrasound.

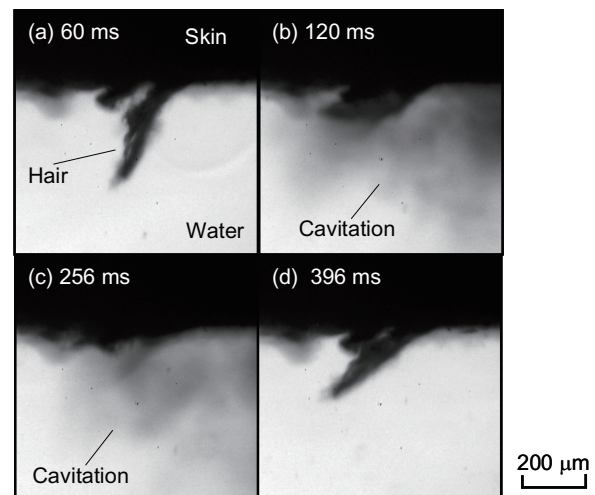


Fig. 3 Observed behaviors of cavitation using high-speed video camera.

4. Summary

It was demonstrated that hydrophilic dye could be introduced into the porcine skin using low frequency ultrasound. The results indicated that the barrier of the skin against the dye was irreversibly altered. Moreover, optical observation of cavitation behaviors shows that the force pressing the skin surface was generated by cavitation. Although it is necessary to investigate the relationship between cavitation and introduction effect, these results contribute to the mechanism of TDD using low-frequency ultrasound.

References

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2. K. Yoshida, S. Nakatani, A. Tsukamoto, T. Ushida and Y. Watanabe: *Jpn. J. Appl. Phys.* **47** (2008) 2400.