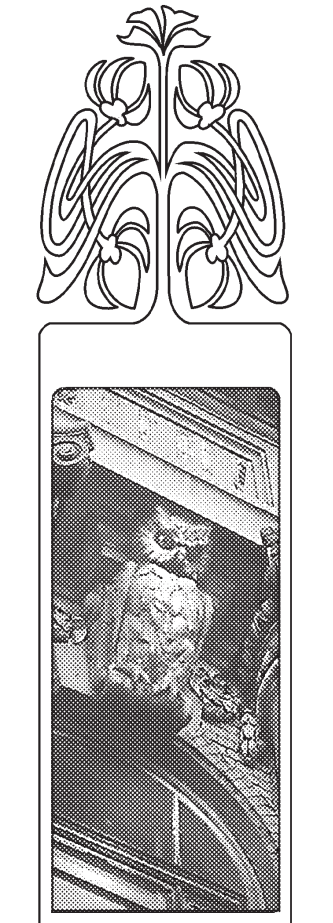

НАУЧНЫЙ ОТДЕЛ

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Sonophoretic acceleration of degradation process for vaterite particles delivered into the hair follicles

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Abstract. Intrafollicular drug delivery is beneficial in terms of both localized therapy of relevant skin disorders and systemic transportation of bioactive molecules. Vaterite particles are capable of loading and delivering various substances to hair follicles. Possibility to control the duration of their intrafollicular degradation can improve such a particulate delivery system. Here, we propose the use of sonophoresis (1 MHz, 1 W/cm^2) to accelerate the resorption of vaterite carriers inside the hair follicles of rats *in vivo*. The effect of sonication is demonstrated utilizing optical coherence tomography monitoring of the skin and scanning electron microscopy investigation of the plucked hairs. A nine-minute post-treatment of skin in the site of particle delivery allowed us to almost halve the time of their degradation.

Keywords: transdermal drug delivery, follicular penetration, calcium carbonate carriers, sonophoresis, particulate formulation, resorption

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Улучшение деградации лекарственных носителей на основе ватерита внутри волосяных фолликулов с помощью сонофореза

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Аннотация. Доставка лекарств в волосяные фолликулы или через них играет важную роль как в локальной терапии кожных заболеваний соответствующей локализации, так и при системной транспортировке биологически активных веществ. Частицы ватерита являются примером инновационного контейнера, применяемого для иммобилизации и интрафолликулярной доставки лекарств. Возможность управления продолжительностью их деградации внутри фолликулов способна открыть перспективы для улучшения такой системы доставки. Данная работа предлагает использование низкоинтенсивного терапевтического ультразвука (сонофореза) частотой 1 МГц для ускорения резорбции ватеритных носителей внутри волосяных фолликулов *in vivo*. Эффект от сонофореза демонстрируется путем исследования волосяных фолликулов и кожи крыс методами оптической когерентной томографии и сканирующей электронной микроскопии. Ультразвуковая пост-обработка (1 Вт/см^2, 9 мин) кожи в месте предварительного внедрения частиц ватерита позволила почти вдвое сократить время их полной резорбции внутри волосяных фолликулов.

Ключевые слова: трансдермальная доставка лекарств, проникновение в волосяные фолликулы, частицы карбоната кальция, сонофорез, резорбция

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Introduction

Hair follicles offer a transportation route for topically applied substances allowing the avoidance of stratum corneum barrier [1–3]. A pilosebaceous unit represents both a targeted site for localized therapy of relevant skin disorders and a pathway for systemic drug delivery [4, 5]. As each delivery mode, follicular transportation requires a suitable balance between effective penetration and safety for the skin. Safe particulate system for intrafollicular delivery should be either biodegradable or able to move out of the follicles in due course.

Recently, we have proposed a biocompatible degradable system based on vaterite particles, which provided effective delivery to the hair follicles and the sustained release of the payload [6]. Topical application of the particles was followed by a therapeutic ultrasound treatment to grant their deep penetration through the skin along the entire depth of the follicle. The system demonstrated total resorption in the skin of rats *in vivo*. The degradation process took 12 days representing a beneficial feature of the system in terms of prolonged intrafollicular storage of bioactive molecules. Potential application of the proposed delivery approach in vitiligo treatment and antifungal therapy was discussed [7–9].

On the other hand, the possibility to control the duration of intrafollicular particle degradation can improve the delivery system. Previously we demonstrated that the use of sonophoresis (0.89 MHz, 1 W/cm^2) during more than 5 min sufficiently affected the process of vaterite particle degradation inducing the release of the payload [10]. Thus, application of such therapeutic ultrasound on the skin surface as a post-delivery treatment may influence the degradation of intrafolliculary delivered particles. 1-MHz ultrasound does not induce adverse effects on skin even when applied at a higher power density (2 W/cm^2) [11, 12]. Thus, such an approach towards the acceleration of vaterite particle degradation in skin reveals non-invasive. Moreover, it can be realized using the same device as applied for intrafollicular introduction of these carriers by increasing a power density and duration of sonication. That is a clear advantage of such method favouring its elaboration.

1. Materials and methods

Materials

Calcium chloride ($CaCl_2$), sodium carbonate (Na_2CO_3) and ethylene glycol (EG) were purchased from Sigma-Aldrich and used without further purification. Milli-Q water was used in all experiments (Milli-Q Purification System, Millipore, Merck, USA).

Particle synthesis

Vaterite particles were synthesized by precipitation from the mixture of equimolar solutions of $CaCl_2$ and Na_2CO_3 at the EG presence in the reaction solution according to previously reported protocol [13].

Animals and ethics statement

Male albino rats of 8 months of age were obtained from Animal Facility of Saratov State Medical University (SSMU). The protocols on animal experiments were approved by the Ethics Committee of SSMU (approval No 8 from 10.04.2018). All the experiments were performed with proper care without pain and suffering of animals in accordance with the principles of bioethics.

Transfollicular delivery of vaterite particles

Delivery of topically applied vaterite particles into hair follicles was performed *in vivo* in rats according to previously developed protocol including the use of sonophoresis as a physical enhancer of the transdermal particle penetration [6]. Dynatron 125 ultrasonicator (Dynatronics, USA) with a frequency of 1 MHz was used for this purpose at 0.5 W/cm^2 of power density during 2 minutes. The amount of vaterite carriers applied to the experimental site was 20 mg.

Penetration of vaterite particles was proven by the means of optical coherence tomography (OCT) monitoring of rat skin *in vivo* and by scanning electron microscopy (SEM) imaging of the hairs plucked with root sheaths from the skin.

OCT-visualization was performed with the use of OCP930SR device (Thorlabs, USA) with the central wavelength of 930 ± 5 nm, spectral full width at half maximum 100 ± 5 nm, the axial resolution in air of 6.2 μm, the lateral resolution of 9.6 μm and the scan area of 2 mm. The treated area was imaged before the particle application and at the end of the delivery procedure.

For the SEM investigation, hairs of the experimental and control (before the particle application) sites were extracted using tweezers retaining their root sheaths and placed then on the conductive tape attached to the sample holder. The root sheaths of these hairs were afterwards destructed mechanically with a cutter knife. The morphology of the samples was investigated using MIRA II LMU instrument (Tescan, Grech Republic) at an operating voltage of 20 kV.

Sonophoretic post-treatment on the delivery site

Additional application of sonophoresis on the site of particle delivery was performed *in vivo* when the particles were successfully delivered into the hair follicles of rats. The same ultrasonicator as for particles delivery was used, however at the higher power density of 1 W/cm^2. Time of sonication was prolonged up to 9 min in order to enhance the cumulative dose of ultrasound energy influencing the process of intrafollicular degradation of the particles.

The influence of this sonophoretic post-treatment on the state of the particles inside the follicles was studied using OCT-monitoring of rat skin *in vivo* and SEM-imaging of the hairs plucked with root sheaths from the skin as described above.

Study of intrafollicular particle degradation *in vivo*

Degradation of the vaterite particles inside hair follicles without any additional treatment and after the sonophoretic post-treatment was monitored using SEM. Accomplishing this, the specimens of hair plucks were collected during 216 hours after the particle delivery and post-treatment procedure.

2. Results and discussion

Being biodegradable, vaterite carriers demonstrate gradual resorption inside hair follicles after their delivery *in vivo*. Duration of this process was found to be dependent on the animal model. In particular, it was shown that degradation of vaterite carriers in mice follicles lasted 120 hours [8]. Meanwhile, their resorption in rat follicles and skin took longer (more than 168 hours) [6, 14]. Such variation can be associated with the species difference in skin metabolism [15, 16]. A search for techniques allowing one to control over the rate of carrier degradation in animal models remains actual. To prolong the vaterite particle resorption, modification of their surface with polyelectrolytes can be used as it provides the stabilizing effect. Acceleration of this process can be achieved by applying the sonophoretic treatment [10].

In order to prove the applicability of such approach for the acceleration of the vaterite carrier degradation inside the hair follicle, sonophoretic post-treatment was applied *in vivo* in rats. Schematics of the process is presented in Figure 1.

Vaterite carriers were topically applied on the lower back of rats and delivered into hair follicles. SEM image of destructed root sheath tissue obtained from the hair plucked after particle delivery (Fig. 2 *b*) indicates an abundant filling of the follicle sac with vaterite carriers. OCT-image of the rat skin in the site of particle delivery (Fig. 2 *e*) confirms their successful intrafollicular incorporation: bright white channels represent the hair follicles filled with particles as they have a higher refraction in-

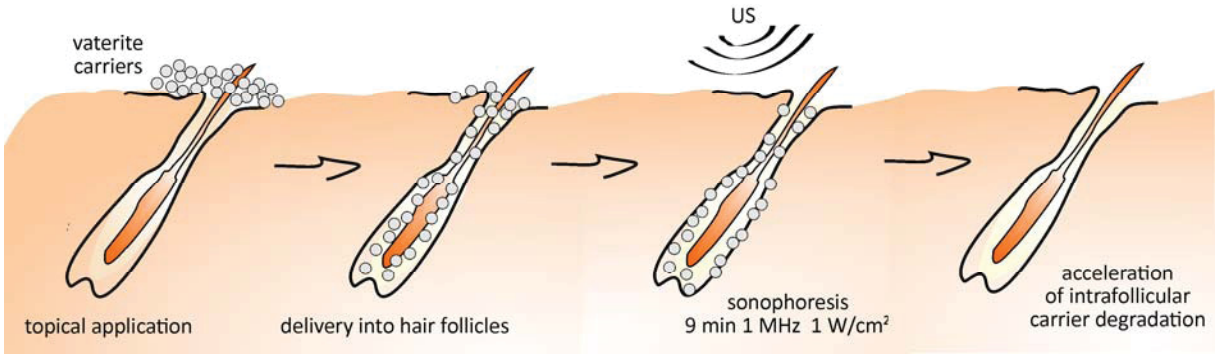

Fig. 1. Schematic illustration of the proposed approach to the acceleration of the vaterite carrier degradation inside hair follicles

dex than surrounding tissue (for vaterite n_o = 1.55 and n_e = 1.65 [17], meanwhile the skin tissue is assumed to have a refractive index of n = 1.37 at 930 nm). The empty hair follicles (Fig. 2 *d*) are less scattering and therefore seen darker than the surrounding.

Sonophoretic post-treatment was applied then on the site of particle delivery. The SEM-image presented in Figure 2 *c* demonstrates drowning of the particles in tissue of a follicle sac after the additional sonication. Meanwhile, in the inner volume of the hair follicle packing of the carriers became less dense. OCT-monitoring has confirmed this observation (Fig. 2 *f*): the middle of the hair follicle volume appeared again darker than the surrounding tissue as they became less scattering, while the follicle walls became brighter and a white follicle-shaped border appeared. That also indicated the particle drowning in follicle walls.

The influence of this sonophoretic post-treatment on the rate of particle degradation inside hair follicles was studied as well. For this purpose, SEM-imaging of the hairs plucked with root sheaths from the rat skin at different time points after vaterite carrier delivery was performed. Experimental skin sites with and without sonophoretic post-treatment were compared and the most significant results are presented in Figure 3.

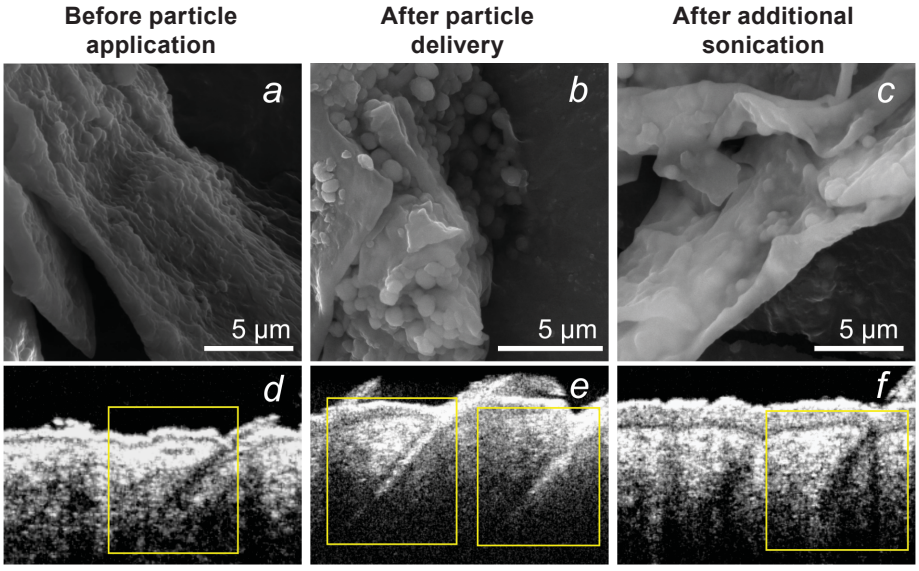

Fig. 2. Influence of sonophoretic post-treatment of skin on the state of vaterite carriers inside the hair follicles. SEM-images of the root sheath fragments of hairs plucked from the skin (*a*–*c*) and OCT-images of rat skin with well-defined hair follicles performed *in vivo* (*d*–*f*). The left-most column of images (*a*, *d*) represents the hair follicles before vaterite carrier application. The second column (*b*, *e*) shows intrafollicular penetration of the carriers at the end of the delivery procedure. The right-hand column (*c*, *f*) demonstrates the result of sonophoretic post-treatment on the site of carrier delivery. Yellow rectangles mark the area of hair follicle localization (color online)

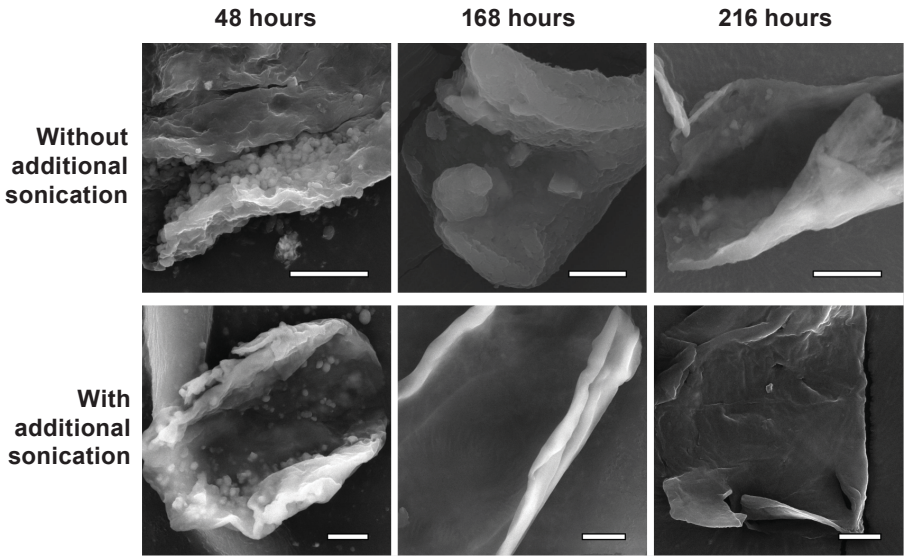

Fig. 3. Influence of sonophoretic post-treatment of skin on the degradation of vaterite particles inside the hair follicles. SEM-images of the root sheath fragments of the hairs plucked from the skin at different time points after the particle delivery. The upper row represents the hairs plucked from the skin site without any additional treatment and the lower row – with sonophoretic post-treatment. Scale bar, 5 μm

It can be seen from the SEM-images, that degradation of vaterite carriers went faster after the sonophoretic post-treatment on the site of particle delivery. Thus, it allows one to reduce the duration of this process in follicles of rats from 12 to 7 days.

Conclusions

The current study proposed a non-invasive and simple approach to the acceleration of vaterite carrier degradation in skin. Application of sonophoretic post-treatment (1 MHz, 1 W/cm^2, 9 min) after their delivery into hair follicles allowed the reducing of degradation period for this system by almost two times. This approach can be effectively applied for the drug-loaded vaterite carriers delivering various bioactive substances to hair follicles. Sonophoresis promotes the faster carrier degradation inducing, therefore, a faster release of a payload. Thus, it allows the controlling of drug release profile whenever it required for the purposes of localized therapy of skin disorders or tuneable systemic transportation of bioactive molecules.

References

1. Knorr F., Lademann J., Patzelt A., Sterry W. U. Blume-Peytavi, Vogt A. Follicular transport route – Research progress and future perspectives. *European Journal of Pharmaceutics and Biopharmaceutics*, 2009, vol. 71, iss. 2, pp. 173–180.
2. Blume-Peytavi U., Vogt A. Human hair follicle: Reservoir function and selective targeting. *British Journal of Dermatology,* 2011, vol. 165, pp. 13–17.
3. Roberts M. S., Cross S. E, Pellett M. A. Skin transport. *Drugs and the Pharmaceutical Sciences*, 2002, vol. 119, pp. 89–196.
4. Wosicka H. , Cal K. Targeting to the hair follicles: Current status and potential. *Journal of Dermatological Science*, 2010, vol. 57, iss. 2, pp. 83–89.
5. Lademann J., Knorr F., Richter H., Jung S., Meinke M. C., Rühl E., Alexiev U., Calderon M., Patzelt A. Hair follicles as a target structure for nanoparticles. *Journal of Innovative Optical Health Sciences*, 2015, vol. 8, no. 4, pp. 1530004.
6. Svenskaya Y. I., Genina E. A., Parakhonskiy B. V., Lengert E. V., Talnikova E. E., Terentyuk G. S., Utz S. R., Gorin D. A., Tuchin V. V., Sukhorukov G. B. A Simple Non-Invasive Approach toward Efficient Transdermal Drug Delivery Based on Biodegradable Particulate System. *ACS Applied Materials & Interfaces*, 2019, vol. 11, iss. 19, pp. 17270–17282.
7. Svenskaya Y. I., Talnikova E. E., Parakhonskiy B. V., Tuchin V. V., Sukhorukov G. B., Gorin D. A., Utz S. R. Enhanced topical psoralen–ultraviolet A therapy via targeting to hair follicles. *British Journal of Dermatology*, 2020, vol. 182, iss. 6, pp. 1479–1481.
8. Gusliakova O., Verkhovskii R., Abalymov A., Lengert E., Kozlova A., Atkin V., Nechaeva O., Morrison A., Tuchin V., Svenskaya Yu. Transdermal platform for the delivery of the antifungal drug naftifine hydrochloride based on porous vaterite particles. *Materials Science and Engineering: C*, 2021, vol. 119, p. 111428.

9. Lengert E., Verkhovskii R., Yurasov N., Genina E., Svenskaya Yu. Mesoporous carriers for transdermal delivery of antifungal drug. *Materials Letter*, 2019, vol. 248, pp. 211–213.
10. Svenskaya Y. I., Navolokin N. A., Bucharskaya A. B., Terentyuk G. S., Kuz'mina A. O., Burashnikova M. M., Maslyakova G. N., Lukyanets E. A., Gorin D. A. Calcium carbonate microparticles containing a photosensitizer photosens: Preparation, ultrasound stimulated dye release, and *in vivo* application. *Nanotechnologies in Russia*, 2014, vol. 9, iss. 7–8, pp. 398–409.
11. Polat B. E., Hart D., Langer R., Blankschtein D. Ultrasound-mediated transdermal drug delivery: Mechanisms, scope, and emerging trends. *Journal of Controlled Release*, 2011, vol. 152, iss. 3, pp. 330–348.
12. Levy D., Kost J., Meshulam Y., Langer R. Effect of ultrasound on transdermal drug delivery to rats and guinea pigs. *The Journal of Clinical Investigation*, 1989, vol. 83, iss. 6, pp. 2074–2078.
13. Parakhonskiy B. V., Haase A., Antolini R. Sub-Micrometer Vaterite Containers: Synthesis, Substance Loading, and Release. *Angewandte Chemie*, 2012, vol. 51, iss. 5, pp. 1195–1197.
14. Genina E. A., Svenskaya Yu. I., Yanina I. Yu., Dolotov L. E., Navolokin N. A., Bashkatov A. N., Terentyuk G. S., Bucharskaya A. B., Maslyakova G. N., Gorin D. A., Tuchin V. V., Sukhorukov G. B. *In vivo* optical monitoring of transcutaneous delivery of calcium carbonate microcontainers. *Biomedical Optics Express*, 2016, vol. 7, iss. 6, pp. 2082–2087.
15. Rittirod T. Species difference in simultaneous transport and metabolism of ethyl nicotinate in skin. *International Journal of Pharmaceutics*, 1999, vol. 178, iss. 2, pp. 161–169.
16. Kao J., Patterson F. K., Hall J. Skin penetration and metabolism of topically applied chemicals in six mammalian species, including man: An in vitro study with benzo [a] pyrene and testosterone. *Toxicology and Applied Pharmacology*, 1985, vol. 81, iss. 3, pp. 502–516.
17. Weber M. J. *Handbook of Optical Materials*. Boca Raton, CRC Press, 2002. 536 p.

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