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Immediate topical drug delivery by natural submicron injectors

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ABSTRACT

Transdermal delivery is an attractive but challenging solution for delivery of drugs. The sea anemone possesses a sophisticated injection system, which utilizes built-in high osmotic pressures. The system is folded within microcapsules and upon activation it injects a long, needle-like tubule of submicron diameter that penetrates the target in a fraction of a second. Here we show that this natural injection system can be adapted for active topical drug delivery once it is isolated from the cells, formulated into a topical gel, and uploaded with the desired drug. The formulated injectors retained their physical characteristics and were capable of penetrating the skin, achieving immediate delivery of a hydrophilic compound. We demonstrate quantitative rapid delivery of lidocaine hydrochloride as a function of microcapsular and drug concentrations. The adaptation of natural injectors for drug delivery combines the benefits of short topical application with rapid delivery of physical devices, thereby presenting a promising alternative for transdermal drug delivery.

1. Introduction

The transdermal route offers a potentially attractive solution for local and systemic drug delivery, provided that certain obstacles can be overcome. The main impediment to efficient drug delivery via this route is the stratum corneum, which serves as an effective barrier to penetration and permeation (Barry, 2004). A variety of vehicles and technologies have been developed in an effort to penetrate this outer layer of skin for the purpose of transdermal drug delivery (Brown et al., 2006; Cevc and Vierl, 2010). The two main approaches employed to enhance skin permeability and penetration are based either on chemical passive enhancers or on physical active or energetic devices. The passive technologies have the advantage of high patient compliance, but they usually require a long application time because of slow absorption and accordingly are adapted for applications where slow and controlled release are desired (Baroli, 2010; Elsayed et al., 2007). The active methods, while allowing rapid penetration and better permeation of the skin barrier, necessitate professional administration and are expensive (Charoo et al., 2010; Ogura et al., 2008; Prausnitz, 2004). Owing to these limitations only a relatively small number of drugs can currently be introduced transdermally (Prausnitz and Langer, 2008; Subedi et al., 2010). We show here that a natural submicron-injection system, isolated from the sea anemone, can serve as an active vehicle for drug delivery in a topical formulation. Although this transdermal delivery is effected by means of an active process, it needs no external power device.

Cnidaria is an aquatic phylum containing thousands of species including sea anemones, corals, jellyfish and hydra. It is one of the most ancient of the multicellular phyla, dating back about 700 million years, and is characterized by the presence of stinging cells. These cells contain cnidocysts, which are microcapsules equipped with a submicron-diameter injection system (David et al., 2008; Tardent, 1995). Within the microcapsule a long, thin tubule is tightly folded in a condensed matrix of short chains of poly-γ-glutamate (pγGlu) and cations (Szczepanek et al., 2002; Weber, 1990). In nature, the cnidocyst is embedded in the cell and is triggered through a cell-sensor mechanism. Upon activation water flows into the microcapsule, causing the pγGlu/cation matrix to dissociate. The resulting osmotic pressure increases to 150 bars, causing the discharge of a long, folded tubule at an acceleration of 5×10^6 *g* (Holstein et al., 1994; Nüchter et al., 2006; Ozbek et al., 2009). This discharge process, one of the fastest events in cell biology, is typically completed within 3 ms (Holstein and Tardent, 1984). About 30 subtypes of microcapsules in Cnidaria have been described. They differ in size, shape and length of tubule, but all function according to the same basic principle. Given these unique physical characteristics, the cnidocyst can potentially serve as an optimal vehicle for hydrophilic drug delivery, with the tubule functioning as the penetrating needle, the microcapsule as the syringe reservoir, and the glutamate matrix as the energy source.

The aim of the present study was to investigate the potential use of this natural system as an active vehicle in transdermal drug delivery. The specific objectives were to demonstrate active rapid

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delivery to the skin by the isolated natural injectors in a topical formulation, and to determine the natural injection parameters compatible with quantitative delivery of lidocaine hydrochloride (HCl). We describe the principle of operation of this injection system for active topical drug application and demonstrate its potential advantages in terms of both rapid delivery and patient compliance.

2. Materials and methods

2.1. Chemicals and reagents

Lidocaine HCl and all reagents were from Sigma–Aldrich (Israel) unless stated otherwise, and were of at least analytical grade. Acetonitrile (HPLC grade) and ethanol were purchased from Riedel de Haen (Seelze, Germany). EMLA® cream (AstraZeneca) was purchased from a local pharmacy. Klucel® HF Pharm hydroxypropylcellulose (HPC) was from Hercules Aqualon (Wilmington, DE, USA).

2.2. Skin

Skins of 8-week-old female nude mice (CD1 strain) were obtained from Harlan Laboratories (Israel). The study was approved by the National Council for Experiments on Animals (Israel). Skin samples, 2 cm × 2 cm, were wrapped in Parafilm®, maintained at −20 °C, and used within 20 days of harvesting.

2.3. Isolation of microcapsules

The sea anemone *Aiptasia diaphana*, when triggered mechanically, secretes thin filaments (acontia) enriched with microcapsules (Fig. 1). We exploited this natural behavior to obtain filaments without harming the anemone. Microcapsules were isolated from the filamentous tissue as described previously (Greenwood et al., 2003; Lotan et al., 1995). Briefly, filaments were incubated in 1 M sodium citrate, followed by two centrifugations in 70% Percoll gradients. The isolated microcapsules were washed with decreasing concentrations of either $CaCl_2$ or NaCl (1–0.2 M) and freeze-dried. The purified microcapsules were kept in the form of a powder until use. Pictures were taken with a Zeiss Axioskop 40 microscope.

2.4. Formulations

The microcapsule powder was mixed with topical gel consisting of 2% HPC in absolute ethanol. We applied different quantities of microcapsules in a constant amount of gel, ranging from approximately $(0.1–1.4) \times 10^6$ microcapsules per square centimeter. The microcapsule gel was used on the day of its preparation.

We tested three concentrations (2.5%, 5% and 10%) of lidocaine HCl at pH values ranging from 5.8 to 6.3 in Millipore Milli-Q® purified water (DDW). The lidocaine HCl solution was prepared every week and kept at 4 °C until use.

2.5. Activation of microcapsules

HPC–ethanol gel containing microcapsules was activated with a hydrophilic solution (DDW, lidocaine HCl, or methylene blue). Activation of the preparation was examined under the microscope. The gel sample was spread over a slide, a drop of DDW was placed over the gel to activate the microcapsules, and the percentage of activated discharged microcapsules was calculated. Only samples in which at least 95% of the microcapsules became activated were used. This high percentage of activation was typical of all the Na^+-based microcapsule preparations utilized and was used as a standard quality control test for these preparations.

2.6. Diffusion cell method

Permeability of the tested compounds through the full skin of a nude mouse was measured *in vitro* in a Franz diffusion cell system (PermeGear V3) at 35–37 °C. The diffusion area was 0.636 cm^2 (9 mm diameter orifice), and the volume of the receiver compartment was 5 ml. The solution on the receiver side was stirred by an externally driven, Teflon-coated magnetic bar. Skin pieces were placed on the receiver chambers with the stratum corneum facing upwards and the donor chambers were clamped in place. Microcapsule powder mixed into 8 mg of gel was introduced into the donor chambers on the skin and overlaid with excess of lidocaine-HCl solution (200 μl). As a control, skin pieces were covered with gel without microcapsules, and overlaid with the same lidocaine HCl solution. After 5 min the lidocaine HCl solution was removed and the embedded skins in the Franz cell apparatus were thoroughly washed 5 times with Millipore DDW. These washes removed the lidocaine HCl residues but did not remove the microcapsules from the skin. The skins were then left in the diffusion cell for up to 24 h to allow the delivered drug to diffuse under the skin. During that period, samples were taken from the receiver compartment after 0.5, 1, 1.5, 3, 5, and 23–24 h and analyzed by high-performance liquid chromatography (HPLC).

Diffusion cell experiments with EMLA® cream were done using 130 mg for 5 min application in a similar way to the microcapsule gel experiments, or for 1 h application as suggested by the manufacturer. The only difference from the microcapsule experimental procedure was that the EMLA® formulation, being an emulsion of oil in water, could not be washed out with DDW, and 0.1% sodium dodecyl sulfate was added to facilitate its removal.

2.7. HPLC analysis

We analyzed the lidocaine content of the various samples using a Hewlett-Packard HP-1090 HPLC system equipped with an autosampler and a diode-array UV detector. The analysis was performed at room temperature with a 5-μm Kromasil Cyano column (KR60-5CN; 250 mm × 4.6 mm) mounted on a guard column (VDS-Optilab, 10 mm × 4 mm Kromasil-5-60 CN cartridge). The mobile phase, consisting of 60% A (80% phosphate buffer 0.02 M, pH 6: 20% acetonitrile) and 40% B (acetonitrile), was pumped at a flow rate of 1.2 ml/min. The injection volume was 40 μl. The UV detector was set at a wavelength of 210 nm. In order to separate the lidocaine from the prilocaine in the EMLA® cream the ratio of the A to B solutions in the mobile phase was changed to 84% A and 16% B and the flow rate was 1.5 ml/min.

2.8. Data

Data were expressed as the cumulative lidocaine permeation per square centimeter of skin surface. Owing to the sampling of large volumes from the receiver solution and their replacement with equal volumes, the solution was continuously diluted. Taking this into account, cumulative drug permeation (Q_t) was calculated from the following equation:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i is the drug concentration of the ith sample, and

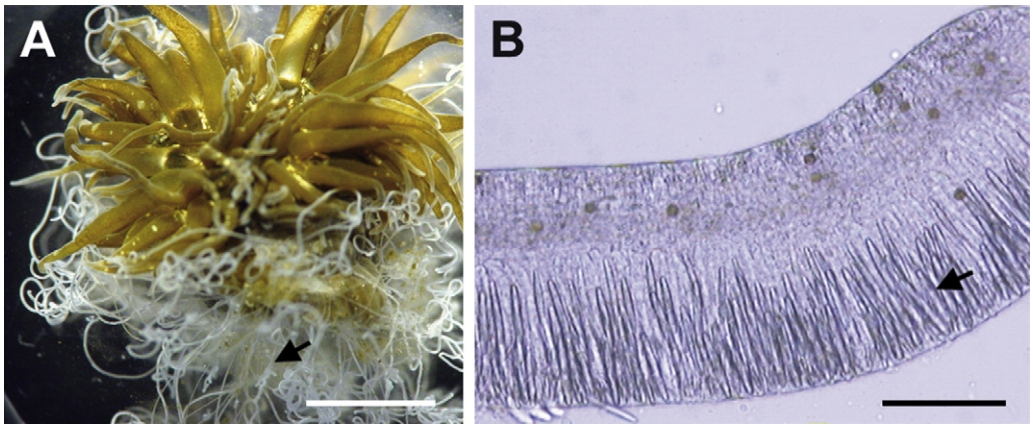

Fig. 1. (A) *Aiptasia diaphana* after release of the white filamentous acontia (arrow). Bar, 1 cm. (B) Part of a filament containing packed cnidocysts (arrow). Bar, 100 μm.

V_r and V_s are the receiver solution and the sample volumes, respectively.

2.9. Statistical analysis

Results are presented as the means and standard deviations of at least eight determinations. Statistical analysis was performed using an unpaired *t*-test. $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Microcapsule characteristics

The secreted filaments of the sea anemone *A. diaphana* were used as a source of the microcapsules to be isolated (Fig. 1). An isolated microcapsule preparation contained one type of intact cylindrical microcapsule, 60 μm in length and 8 μm in diameter (Fig. 2A). In their natural state the cnidocysts are kept intact within cells until their discharge is triggered by a complex cascade. In order to utilize cnidocysts as a delivery device we needed (i) to find a way to preserve and stabilize the isolated microcapsules in their intact resting state, and (ii) to select a trigger that would not interfere with the drug to be delivered. These two requirements were taken into consideration when we designed the isolation procedure.

Since isolated cnidocysts are known to be unstable in water, once the microcapsules were isolated they were lyophilized. However, before the microcapsules were dried, their cationic content was changed from Ca^{++} to Na^{+} ions. This was done because the affinity of divalent cations for the anionic pγGlu matrix in the microcapsules is 50–100-fold greater than that of monovalent cations (Szczepanek et al., 2002; Weber, 1989). We therefore assumed that the use of monovalent rather than divalent cations would facilitate the triggering of microcapsules. Moreover, the internal osmotic pressure has been shown to be higher in the presence of monovalent than of divalent cations (Szczepanek et al., 2002; Weber, 1989), suggesting that the activation cascade would be faster. Previous studies have demonstrated that isolated cnidocysts can be activated by a variety of chemicals and enzymes. Each of these triggers, however, might be capable of interacting with the drug to be delivered, and therefore could not be used. The best trigger would be water, as it is the natural carrier of hydrophilic drugs. To examine whether the purified microcapsule

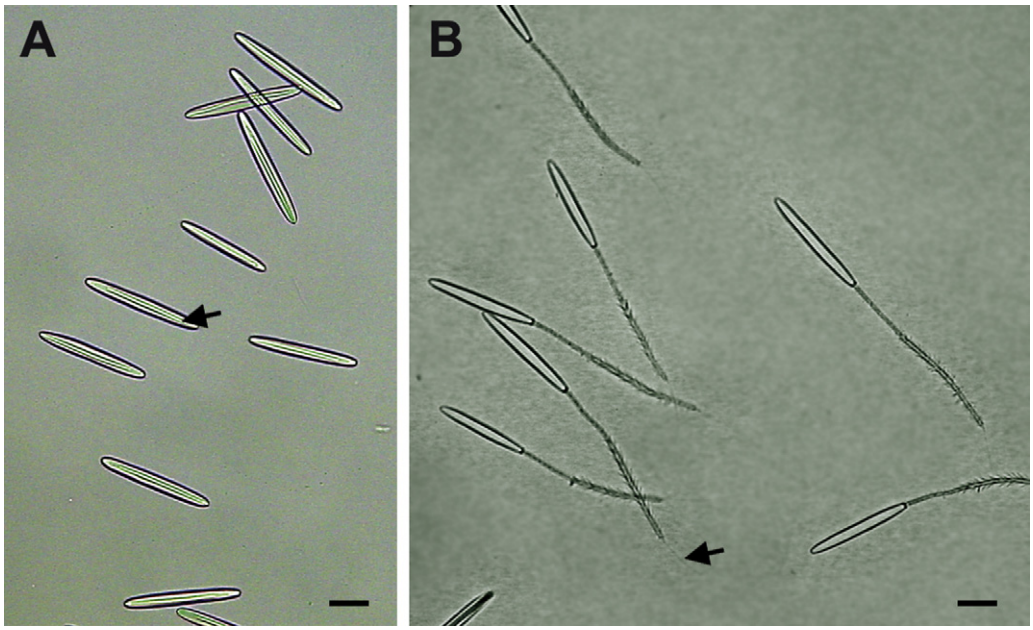

Fig. 2. Sample of isolated cnidocysts. (A) Cnidocysts in a resting state before discharge. The arrow points to the folded injector inside the microcapsule. Bar, 25 μm. (B) Discharged cnidocysts after exposure to DDW. Folded 150-μm injectors are released from microcapsules. The arrow points to the smooth tip (50 μm long, submicron diameter). Bar, 25 μm.

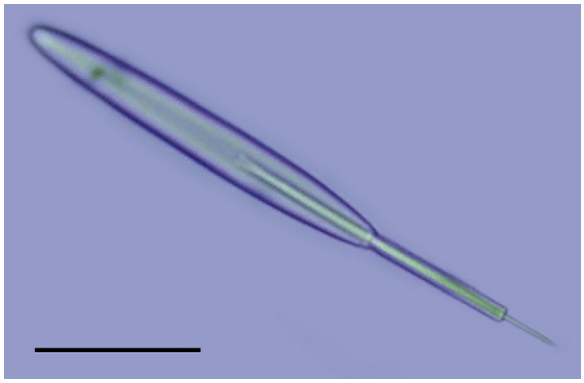

Fig. 3. Live video of discharging microcapsules. Gel containing microcapsules is overlaid with DDW, triggering immediate discharge of injectors. The video is shown in real time (2 s) and in 4× slow motion. The cover figure shows a microcapsule during the discharge process. Bar, 25 μm.

powder had retained its potential for discharging, we mixed it with HPC–ethanol gel, spread it over a microscopic slide, and tested its activation with DDW for up to 5 min. As shown in Figs. 2B and 3 (live video) the microcapsules immediately discharged, releasing a 150-μm-long injector, thus verifying that the system had retained its natural discharge characteristics and was sensitive to hydrophilic triggering. This rapid mode of activation was common to all microcapsule preparations tested with Na^+ cations. With dried Ca^{++} microcapsule preparations, however, large variations were found between preparations, and in order to obtain microcapsule activation in excess of 95% longer activation times were needed and in many cases the release of the injectors was slow and done in two steps. These results are consistent with the higher affinity of Ca^{++} for the pγGlu matrix, resulting in slower activation and acceleration of the injector. Because we were interested in rapid homogenous activation, all further experiments were carried out with the Na^+-based microcapsule preparations only.

In nature, the injectors penetrate hard substrates such as plankton cuticle and fish scales, and isolated microcapsules can penetrate keratin substrates such as hair or nails (Lotan, 2008). To examine whether isolated microcapsules can penetrate the skin and also deliver an exogenous compound transcutaneously, we applied the gel formulation containing the microcapsules over the skin of a nude mouse *in vitro* and activated it with methylene blue, a hydrophilic dye. After 5 min the skin was rinsed with DDW and photographed. After the rinsing the injectors were retained within the skin, as can be seen in Fig. 4. The results showed that the tested skin turned blue only at the points at which the tubules penetrated the skin and that this coloration did not wash away. Untreated domains, in contrast, remained clear. These findings suggested that the dye had been delivered transcutaneously (Fig. 4). Penetration of the skin was minimal in comparison to that obtained with microneedles 25–200 μm in diameter, or by sono/electroporation, which is more invasive (>200 μm pore diameter) (Cevc and Vierl, 2010; Donnelly et al., 2010). The injector is composed of two main parts: a 100-μm-long shaft containing barbs and a 50-μm-long smooth tubule with a diameter smaller than 1 μm. The skin was penetrated only by the smooth thin tubule (Fig. 4B), indicating that the application was virtually noninvasive. We concluded that isolated microcapsules in gel formulation can be activated for immediate delivery of exogenous hydrophilic compounds to the skin by means of a brief topical application of up to 5 min. Thereafter the gel can be wiped away, as the delivery process has been completed.

3.2. *In vitro lidocaine HCl permeation across nude mouse skin*

To quantify the delivery parameters of the microcapsule gel we tested it using lidocaine HCl as a hydrophilic model drug. Lidocaine is mostly used in its nonhydrophilic phase as a topical formulation for local anesthesia, but because of passive permeation there is a delay of 60–90 min until onset of action. The submicron injection system used in the present study was examined for its ability to achieve immediate active transcutaneous delivery of lidocaine HCl in its hydrophilic phase. Permeation across the full skin of a nude mouse was tested with 5% lidocaine HCl and gel containing 0.95×10^6 microcapsules per square centimeter. The duration of lidocaine HCl application was limited to 5 min as the system had already been shown (with methylene blue) to be activated immediately. The application was followed by washing of the upper skin surface to remove any drug traces, and the skin was left in the diffusion cell for an additional 24 h to allow the delivered lidocaine to diffuse from the skin to the lower reservoir (under the skin) for analysis. The results showed that the amount of lidocaine

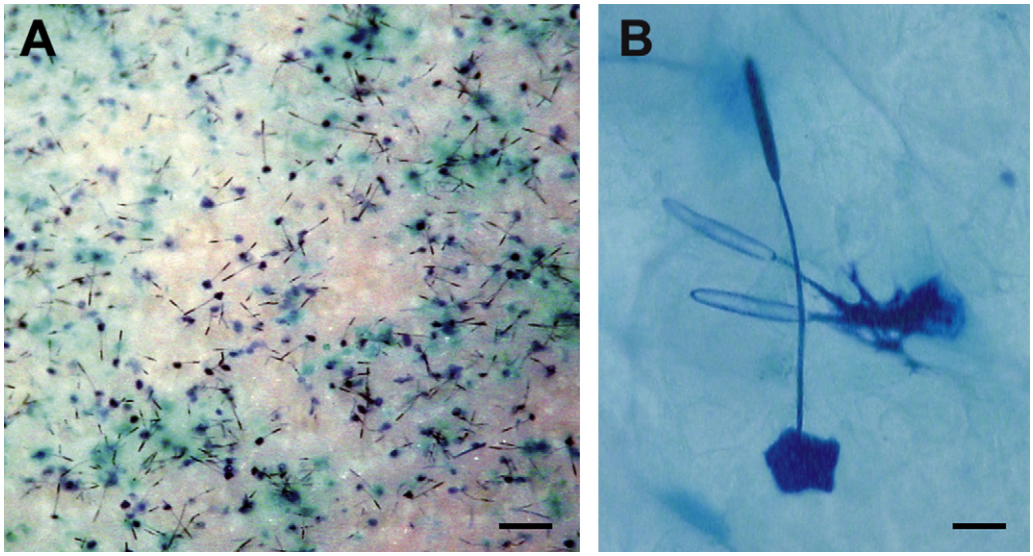

Fig. 4. Delivery of dye compound to the skin. (A) Gel containing microcapsules was spread over nude mouse skin and activated with 0.5% methylene blue solution for 5 min. After rinsing of the skin with water, blue spots in the skin are clearly seen. Bar, 200 μm. (B) Enlargement of one penetrating cnidocyst. A blue spot of delivered dye is seen at the penetration point. Only the 100-μm-long injector is visible as the ultra-thin (submicron diameter) tubule has penetrated the skin and is not seen. Bar, 25 μm.

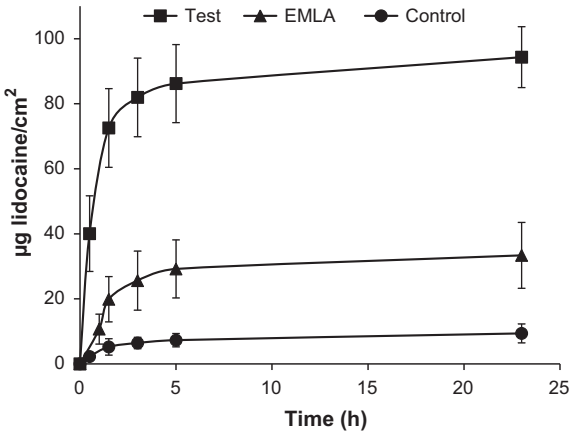

Fig. 5. Kinetics of cumulative lidocaine under the skin. The tested cnidocyst formulation containing 0.95×10^6 microcapsules per square centimeter of skin ($n = 12$), or control formulation without microcapsules ($n = 8$) was spread over the skin and 5% lidocaine HCl solution was added. A commercial emulsion of EMLA® cream ($n = 12$) was tested after application for 5 min as a reference. After 5 min the lidocaine HCl solution and the EMLA® cream were removed, and the skins were washed thoroughly and then left in the diffusion cell for up to 24 h to allow subcutaneous diffusion of the delivered drug. Error bars represent the mean ± SD. The amount of delivered lidocaine was significantly greater in the skin treated with microcapsule gel formulation than in EMLA® cream-treated samples or in control skin samples treated with drug formulation without microcapsules ($P < 0.005$).

delivered transcutaneously was 10 times greater in skin treated with the microcapsule gel formulation than in control skin samples treated with the drug formulation without microcapsules (94 ± 3 compared to 9 ± 1 µg lidocaine/cm^2; Fig. 5). Comparison of these results to those obtained with EMLA® cream (a 5% commercial emulsion preparation containing an eutectic mixture of lidocaine 2.5% and prilocaine 2.5% in a nonhydrophilic state), we found that application of EMLA® for 5 min resulted in about 3 times more lidocaine (33 ± 10 µg lidocaine/cm^2; Fig. 5) than in the control, and about 3 times less than with application of the injectors. Examination of the kinetics of percutaneous penetration by the injectors showed that more than 40% of the delivered drug had already reached the lower compartment under the skin by 30 min after application and more than 90% at 5 h. The rate of delivery of EMLA® was substantially slower, with only 30% of the lidocaine delivered after 1 h. These results are consistent with the active mode of penetration by the injectors relative to the slow passive diffusion of an emulsion through the skin. When EMLA® cream was applied for 1 h, the amount of lidocaine delivered after 24 h was more than 5 times the amount delivered after its application for 5 min (172 ± 34 µg lidocaine/cm^2; $n = 11$), which is 1.8 times greater than the amount delivered after a 5-min application of the injectors.

In a physiological study carried out *in vivo* in rabbits (Lotan, 2005), the microcapsule gel treatment achieved local anesthesia in a similar manner to EMLA®. However, whereas the EMLA® emulsion had been applied to the rabbit skin 1 h prior to the anesthesia test (as instructed by the manufacturer), the injectors had been applied only 4 min before onset of anesthesia.

3.3. Dose-dependent microcapsule/lidocaine HCl experiments

In theory, if the microcapsules serve as miniature drug delivery device compartments, increasing the number of microcapsules on the skin would increase the number of penetrations and should therefore increase the amount of lidocaine in the skin. To examine the effect of the number of microcapsules on lidocaine accumulation, we tested a range of microcapsule concentrations between 0.1×10^6 and 1.4×10^6 microcapsules per square centimeter of skin treated with 5% lidocaine HCl. As in the previous experiment, the

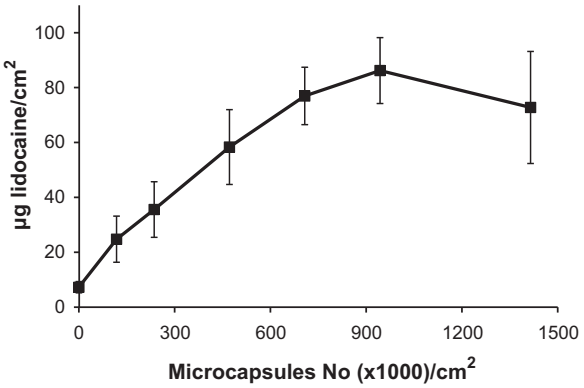

Fig. 6. Accumulation of delivered lidocaine as a function of microcapsule number. Lidocaine HCl (5% solution) was added to gel formulations containing between 0.1×10^6 and 1.4×10^6 microcapsules per square centimeter of skin or to the same gel formulation without microcapsules as a control (0 microcapsules). After exposure to lidocaine HCl for 5 min the drug was removed and the skins were left in the diffusion cell for 5 h to allow subcutaneous diffusion of the delivered lidocaine. Error bars represent the mean ± SD.

system was exposed to lidocaine HCl for 5 min, the drug was then removed, and the amount of lidocaine under the skin was measured for up to 5 h. The results showed (Fig. 6) that the amount of drug delivered was proportional to the number of microcapsules up to approximately 1×10^6 microcapsules per square centimeter of skin. Above this concentration there was a reduction in the amount of drug delivered. As the microcapsules are randomly oriented over the skin ridges and grooves, and since their skin penetration is dependent on their close contact within the skin, their increasing density probably resulted in an overload, preventing their optimal contact with the skin.

In additional experiments, conducted to test the effects of lidocaine HCl concentrations on microcapsule delivery, the number of microcapsules was kept constant at 0.95×10^6 and lidocaine HCl was applied at three concentrations: 2.5%, 5% and 10%. Increasing the concentration of the drug was followed by an increase in the concentrations of lidocaine delivered through the skin. When the concentrations of lidocaine HCl were increased from 2.5% to 5% an approximately proportional 2-fold increase in the amount of drug delivered was observed, whereas a further increase in drug concentration to 10% yielded an increase of only 1.5-fold relative to the amount delivered with the 5% drug preparation (Fig. 7). In control samples only negligible amounts were delivered. A similar pattern

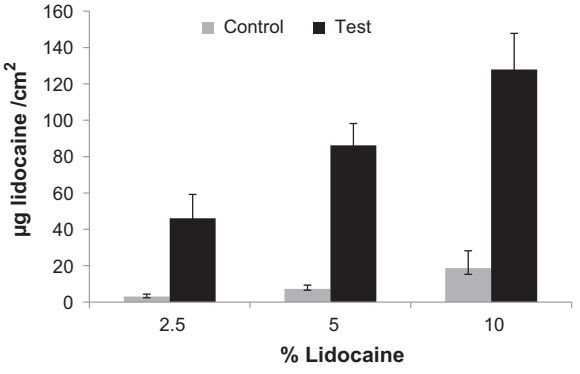

Fig. 7. Cumulative permeation of lidocaine delivered by cnidocyst gel (0.95×10^6 microcapsules per square centimeter) or by control gel without microcapsules, as a function of drug concentration. Lidocaine HCl solutions (2.5%, 5% or 10%) were applied over the tested and control formulations ($n = 8$–13). After exposure to lidocaine HCl for 5 min the drug was removed and the skins were left in the diffusion cell for 5 h to allow subcutaneous diffusion of the delivered lidocaine. Error bars represent means ± SD. All 3 concentrations are statistically significant ($P < 0.05$).

was obtained when the experiment was repeated with a different fixed number of microcapsules that was kept constant in each case. For example, the use of fewer capsules (0.7×10^6) with 2.5%, 5% and 10% lidocaine HCl resulted in 42 ± 10, 77 ± 10, and 120 ± 24 μg lidocaine/cm^2 respectively. The nonlinear drug delivery observed with the 10% lidocaine HCl concentration might imply that the system becomes saturated with the high drug concentration and hence less efficient. These experiments confirmed that the microcapsule gel can actively deliver lidocaine HCl through the stratum corneum within an application time as short as 5 min, and that the amount of hydrophilic drug delivered can be controlled by changing either the number of microcapsules per skin area or the drug concentration.

Unlike other relatively rapid applications, the system presented in this work can be applied by the user without the need for external devices or energy. Moreover, safety experiments in which single-patch dermal irritation and repeated (10×) insult patch sensitization tests were assessed in more than 100 volunteers (Lotan, 2005) yielded no responses indicative of contact dermal irritation or sensitization in any of the testees. In these dermatology tests the injectors were deliberately left in the skin, even though in previous *in vivo* experiments in swine we had found only traces of microcapsules after 2 days, and gentle wiping of the treatment site with gauze after the application had sufficed to remove both microcapsules and injectors from the skin (Lotan, 2008).

3.4. Mode of operation

The microcapsule wall is a porous net permitting free movement of solutions (Lubbock and Amos, 1981), whereas a large matrix of pγGlu and a high concentration of cations are trapped within the microcapsule. The pγGlu matrix is the main substance of the capsule, comprising more than 80% of its content and associates with up to 2 M cations (Weber, 1990). The matrix serves as a built-in "battery" that generates the high osmotic pressure during the discharge. The isolated lyophilized cnidocysts evidently retain their ability to discharge when exposed to hydrophilic solutions. In the intact resting state, when the microcapsules are mixed with the HPC–ethanol gel, there is a balance between the gel medium and the content of the microcapsules; thus, the pγGlu/Na^+ aggregate is stable (Fig. 8A). Upon hydration, however, this balance is upset as water penetrates the microcapsules and the water protons compete for the OH^- residues of the pγGlu. As water is in excess, Na^+ cations are released and the pγGlu interacts with water protons. Dissociation of cations from the pγGlu results in dissociation of the pγGlu aggregate, leading to an increase in the number of particles, which in turn provides the driving force for the increase in microcapsular osmotic pressure, and the resulting high water influx leads to release of the tubule at ultra-high speed. Moreover, as the microcapsule wall is not selective, if the surrounding medium contains soluble drug molecules the drug will be pumped together with the water influx into the microcapsule and will be injected through the tubule into the skin (Fig. 8B). Lidocaine HCl, being a relatively small hydrophilic molecule, can easily penetrate the microcapsule net wall, in the same manner as the methylene blue dye (Fig. 4). We suggest that the osmotic system which activates the discharge mechanism and continues to pump the solution surrounding the microcapsule until all the particles are injected out of the microcapsule also controls the pumping and delivery of the hydrophilic drug. However, we cannot exclude the possibility that the flow results in a low pressure in the injector, dragging additional surrounding drug into the "jet" in accordance with Bernoulli's principle. It is also possible that once the injector penetrates the skin, the lidocaine HCl can diffuse passively through the opened channels in the skin.

The operation of the microcapsule injection system is limited to the use of hydrophilic drugs, as activation of the system is water-dependent and is based on osmotic regulation. Hydrophilic low-molecular-weight compounds were shown here to be delivered by the injection system, but it remains to be investigated whether larger molecules, such as polypeptides and proteins, can be injected in a similar way to lidocaine HCl and have a similar effect. The main limitation for protein delivery would be the small size of the porous net of the microcapsular wall. During discharge, however, the volume of the microcapsule increases by 20–50%, depending on the cnidocyst species (Holstein and Tardent, 1984), and the porous wall stretches. This might be sufficient to facilitate the delivery of short-chain polypeptides, but for larger molecules the microcapsule wall may need to loosen more. The cnidocyst wall is composed mainly of two small proteins that are highly enriched in cysteine residues and are cross-linked by disulfide bonds during microcapsule assembly (Engel et al., 2001). The use of mild detergents or reducing agents can partially open the disulfide bonds supporting the microcapsule wall, and hence enlarge the wall's pores. However, if this process takes place during activation it might impair the protein's function. Further experimental work is needed in order to prepare a modified microcapsular wall that will not weaken the discharge process.

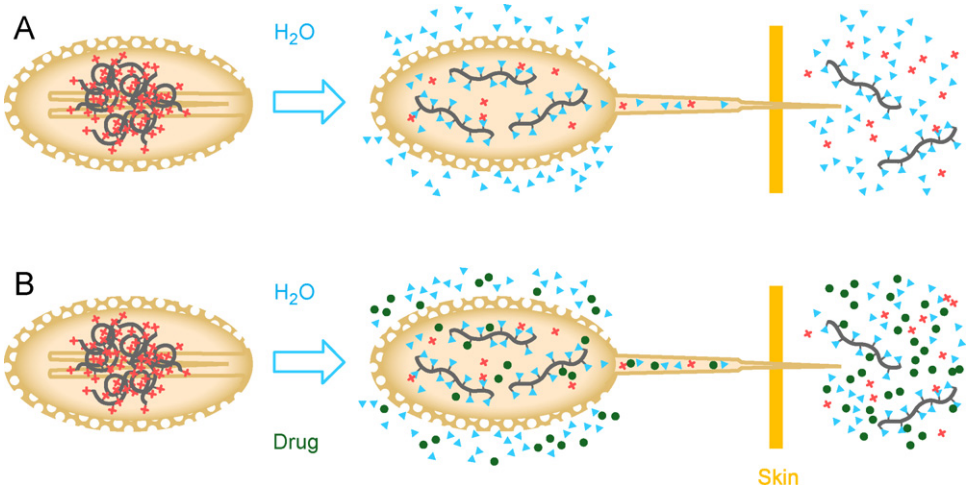

Fig. 8. Model of the cnidocyst as a tool for drug delivery. The model illustrates both the resting (intact) state and the discharge state (penetration of the skin by the tubule). In the intact state the isolated cnidocyst is dry and contains pγGlu with Na^+ (red, +) aggregate. Upon activation (A), water molecules penetrate the porous wall (blue triangle) and the pγGlu/Na^+ aggregate dissociates, resulting in discharge of the tubule and injection of its microcapsule content into the skin. When the hydrophilic solution contains a soluble drug (B), a similar process occurs and the drug (green circles) is delivered with the water through the porous wall to the microcapsule and through the tubule to the skin.

4. Conclusion

The results of this study show that natural isolated cnidocysts can be formulated for topical drug delivery application. The system was applied here as a gel and was compatible with low-molecular-weight hydrophilic molecules. In contrast to standard transdermal delivery applications, upon activation the microcapsules acted immediately to deliver the drug transcutaneously. This delivery system is capable of delivering the drug with minimal penetration of less than 1 μm diameter and up to 50 μm length, suggesting a nearly noninvasive application. Delivery of the tested lidocaine HCl drug was controlled by microcapsule number and drug concentration. The rapid delivery and ease of use offer a promising option for active topical drug delivery. The system can be self-applied, and as the delivery is immediate there is no need to maintain the drug over the skin. Further studies should be designed to investigate the feasibility of using this nature-based technology for the delivery of hydrophilic macromolecules such as proteins.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.07.042.

References

Baroli, B., 2010. Penetration of nanoparticles and nanomaterials in the skin: fiction or reality? J. Pharm. Sci. 99, 21–50.
Barry, B.W., 2004. Breaching the skin's barrier to drugs. Nat. Biotechnol. 22, 165–167.
Brown, M.B., Martin, G.P., Jones, S.A., Akomeah, F.K., 2006. Dermal and transdermal drug delivery systems: current and future prospects. Drug Deliv. 13, 175–187.
Cevc, G., Vierl, U., 2010. Nanotechnology and the transdermal route: a state of the art review and critical appraisal. J. Control. Release 141, 277–299.
Charoo, N.A., Rahman, Z., Repka, M.A., Murthy, S.N., 2010. Electroporation: an avenue for transdermal drug delivery. Curr. Drug Deliv. 7, 125–136.
David, C.N., Ozbek, S., Adamczyk, P., Meier, S., Pauly, B., Chapman, J., Hwang, J.S., Gojobori, T., Holstein, T.W., 2008. Evolution of complex structures: minicollagens shape the cnidarian nematocyst. Trends Genet. 24, 431–438.
Donnelly, R.F., Singh, T.R.R., Woolfson, A.D., 2010. Microneedle-based drug delivery systems: microfabrication, drug delivery, and safety. Drug Deliv. 17, 187–207.
Elsayed, M.M.A., Abdallah, O.Y., Naggar, V.F., Khalafallah, N.M., 2007. Lipid vesicles for skin delivery of drugs: reviewing three decades of research. Int. J. Pharm. 332, 1–16.
Engel, U., Pertz, O., Fauser, C., Engel, J., David, C.N., Holstein, T.W., 2001. A switch in disulfide linkage during minicollagen assembly in Hydra nematocysts. EMBO J. 20, 3063–3073.
Greenwood, P.G., Balboni, I.M., Lohmann, C., 2003. A sea anemone's environment affects discharge of its isolated nematocysts. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 134, 275–281.
Holstein, T., Tardent, P., 1984. An ultrahigh-speed analysis of exocytosis: nematocyst discharge. Science 223, 830–833.
Holstein, T.W., Benoit, M., Herder, G.v., Wanner, G., David, C.N., Gaub, H.E., 1994. Fibrous mini-collagens in hydra nematocysts. Science 265, 402–404.
Lotan, T., 2005. Natural nano-injector as a vehicle for novel topical drug delivery. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption Drugs–Cosmetics – Mechanisms–Methodology. , 4th ed. Taylor & Francis, pp. 521–528.
Lotan, T., 2008. Immediate topical drug delivery using natural nano-injectors. In: Rathbone, M.J., Hadgraft, J., Roberts, M.S., Lane, M.E. (Eds.), Modified-Release Drug Delivery Technology. , 2nd ed. Informa Healthcare, New York, pp. 395–404.
Lotan, A., Fishman, L., Loya, Y., Zlotkin, E., 1995. Delivery of a nematocyst toxin. Nature 375, 456–460.
Lubbock, R., Amos, W.B., 1981. Removal of bound calcium from nematocyst contents causes discharge. Nature 290, 500–501.
Nüchter, T., Benoit, M., Engel, U., Ozbek, S., Holstein, T.W., 2006. Nanosecond-scale kinetics of nematocyst discharge. Curr. Biol. 16, R316–R318.
Ogura, M., Paliwal, S., Mitragotri, S., 2008. Low-frequency sonophoresis: current status and future prospects. Adv. Drug Deliver. Rev. 60, 1218–1223.
Ozbek, S., Balasubramanian, P.G., Holstein, T.W., 2009. Cnidocyst structure and the biomechanics of discharge. Toxicon 54, 1038–1045.
Prausnitz, M.R., 2004. Microneedles for transdermal drug delivery. Adv. Drug Deliver. Rev. 56, 581–587.
Prausnitz, M.R., Langer, R., 2008. Transdermal drug delivery. Nat. Biotechnol. 26, 1261–1268.
Subedi, R., Oh, S., Chun, M.-K., Choi, H.-K., 2010. Recent advances in transdermal drug delivery. Arch. Pharm. Res. 33, 339–351.
Szczepanek, S., Cikala, M., David, C.N., 2002. Poly-{gamma}-glutamate synthesis during formation of nematocyst capsules in Hydra. J. Cell Sci. 115, 745–751.
Tardent, P., 1995. The cnidarian cnidocyte, a hightech cellular weaponry. Bioessays 17, 351–362.
Weber, J., 1989. Nematocysts (stinging capsules of Cnidaria) as Donnan-potential-dominated osmotic systems. Eur. J. Biochem. 184, 465–476.
Weber, J., 1990. Poly(gamma-glutamic acid)s are the major constituents of nematocysts in Hydra (Hydrozoa, Cnidaria). J. Biol. Chem. 265, 9664–9669.