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Review Article

The Role of Cnidocytes in Transdermal Drug Delivery: A Systematic Review

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Abstract:

Cnidaria is a water-dwelling phylum characterized by a cnidocyte stinging cell. In nature, cnidocytes are used to immobilize or "sting" prey, for defense, and for locomotion, but their mechanisms also hold implications for drug delivery. Oral drug delivery has limitations that warrant new drug delivery techniques. One prominent method, transdermal drug delivery, uses the skin as a drug administration platform. Drugs can be systemically absorbed through microcirculation after relatively less invasive, painless, and self-administered delivery through ointments, creams, patches, and microneedles. Research has shown microneedle technology (essentially arrays of miniature needles) could implement cnidarian cnidocytes to bypass current microneedle restraints. Although positive results support cnidocyte gel-based drug delivery, limited variety formulas, conditions, and drugs have been tested, warranting future research before widespread implementation.

Keywords: Cnidocyte, Transdermal drug delivery, Microneedles.

Introduction

In the United States, there are over 19,000 drugs approved for marketing by the FDA, and each year 1 billion therapeutic drugs are prescribed. The foundation of all these drugs, as well as the rest of human disease treatment, stems from animal, plant, and mineral products [1]. From ancient Indians, Chinese, and Egyptians using plants to cure illness 5000 years ago, to the isolation of morphine from Papaver somniferum plant in 1803, the search for medicinal plants is prominent in traditional and modern medicine.

While terrestrial plants hold solutions to many human diseases, a lack of attention is given to marine plants, and even less to marine animals. Oceans make up 71% of our planet's surface and have extreme biodiversity with over 15 endemic phyla [2]. One phylum in particular, Cnidaria, includes sea anemones, corals, and jellyfish, all of which have cnidocytes, stinging cells used to inject toxins. With an approximate time of 700 nanoseconds, cnidocyte discharge acts as one of nature's fastest processes [3].

Cnidocytes are used to immobilize prey and defend from predators, but their mechanisms hold implications for drug delivery. The goal of this literature review is to establish if research on cnidocytes as a method of transdermal drug delivery should be further explored when considering modern transdermal drug delivery advances.

Methodology

I conducted a systematic search of the literature using Google Scholar and the keywords 'cnidocyte' and 'transdermal drug delivery'. Within reviewed citations, I examined the source references for inclusion as well. I only included English language articles in this search.

Cnidaria and Cnidocytes

Cnidaria:

Cnidaria is a 600 million-year-old phylum consisting of approximately 10,000 fresh- and saltwater species [4]. This ecologically diverse phylum has 5 classes and can be found in a variety of aquatic environments [4]. All cnidarians are characterized by having a cnidocyte stinging cell and bilateral symmetry [5, 6]. They exist in a polyp phase (a sessile stage) and/or a medusa phase (a typically free-floating stage). Cnidarian vulnerability and their inability to select prey warrants the need for cytotoxic, cardiotoxic, and neurotoxic effects for access to a larger variety of prey [7]. As a result, cnidarians have been found to consume zooplankton, fish larvae, and even one another. Their venoms make it easier to consume prey and deter predators, but efficient delivery can be difficult. In fact, cnidarians have succeeded as a

predatory phylum because of the complicated and effective structure of their cnidocytes [8].

Cnidocytes in Nature and Mechanisms:

Cnidarians cnidocytes stinging cells are arguably the most complex of all eukaryotic cells [9]. They can be broken down into three distinct types: spirocysts, ptychocysts, and nematocysts. Spirocysts are found in the anthozoa class and immobilize prey. Ptychocysts are found in cerianthid anemones, and serve to construct a surrounding tube that acts as shelter. These could be a different type of cnidocyte or a hyper-specialized nematocyst [10]. Nematocysts are found in all cnidarians with the purpose of prey capture, defense, or locomotion. This type of cnidocyte has been explored for transdermal drug delivery purposes.

Nematocyst is the most diverse group of cnidocytes as they can be found in all classes of Cnidaria, most commonly in their tentacles. Needed for prey capture, defense, and locomotion, some nematocysts adhere to prey, but most also inject venom. They are essential for survival, but their single-use warrants their continued and rapid production. In fact, out of the 11,000 cells in the *Hydra magnipapillata's* polyp form, around 30% are nematocysts and nematoblasts (cells that produce nematocysts) suggesting the essential role of nematocysts in cnidarians [11].

Each species typically has multiple kinds of cnidocytes [12]. It assumed each type of cnidocyte has different functions and there could be correlation between cnidocyte type and diet [12]. For example, the cnidocytes and diet of the Australian Box Jellyfish (*Chironex fleckeri*) change throughout its life cycle in unison [13].

Generally, nematocyst creation starts when an interstitial cell turns into a cnidoblast [14]. It is followed by the creation of the capsular region and tube [14]. Once mature, the nematocyst vesicle is in the apical side of the cell and can be found in the epithelial cell layer [10]. Structurally, the nematocyst is a variety of protein species that forms a double-walled capsular structure which is made of a relatively thick elastic substance [10]. The matrix is made up of short chains of poly-γ-glutamate that binds a 2M concentration of cations [15]. Inside the capsule is a long spiny tubule, or thread, that is highly folded [10]. Once discharged, it will leave through the lid, or operculum, and often will inject and invert to deliver venom (Fig. 1).

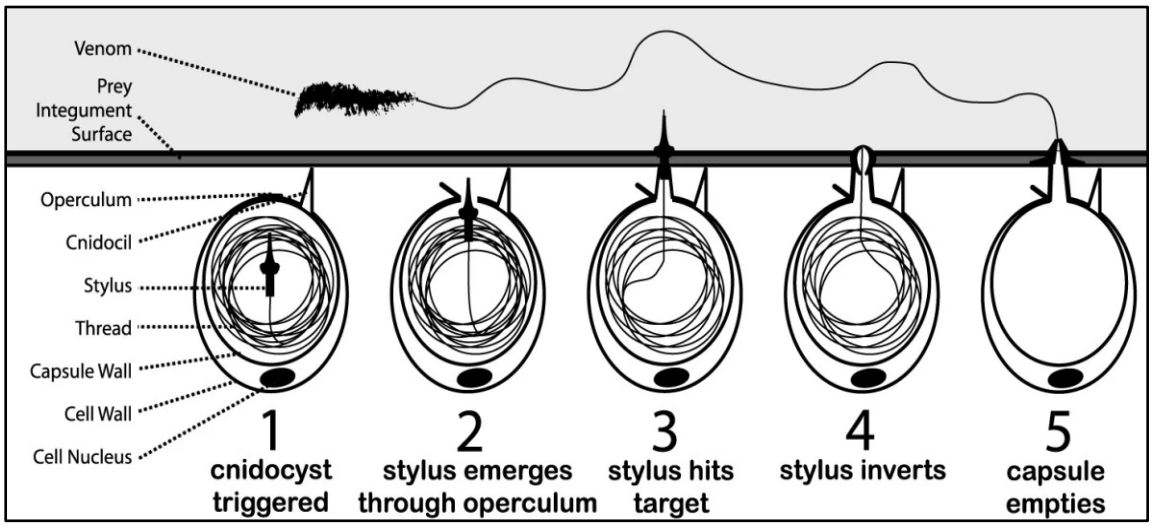

Figure 1: Cnidocyte structure and discharge. Sourced from Oppegard et al. 2009 [20].

There are two leading hypotheses to explain how energy is stored and released for discharge. One explanation is the intrinsic force hypothesis, stating that energy is stored in capsule walls from cnidocyte formation [16]. The opposing osmotic theory states that there is an increase in intracellular osmotic pressure during discharge. This results from sea water causing the poly-y-glutamate and cations to dissociate. Osmotic pressure can increase to 150 bars (15,000 kPa) and may force the tubule to discharge [17].

Cnidarian Species Studied for Drug Delivery:

All cnidarians utilize venom, cnidocytes, and unique body composition to predate and defend themselves. Transdermal drug delivery studies have used the cnidocytes of three species: Portuguese Man O' War jellyfish (*Physalia physalis*), *Aiptasia diaphana* sea anemone, and Starlet sea anemone (*Nematostella vectensis*). The Portuguese Man O' War has an air bladder that enables it to float in the ocean and drag behind deadly tentacles that capture prey [18]. Tentacular palpoons, or tentacle-bearing polyps, are unique to this species and specialize in producing nematocysts [19]. Physalia physalis are able to puncture fish scales with nematocysts that

have long threads, a quality perfect for penetration [19, 20]. As a sessile cnidarian, the *Aiptasia diaphana* relies heavily on defensive venoms for survival. Nematocysts can be found in tentacles and, as in most anemones, are abundant [21]. They are continuously discharged for feeding, defense, and locomotion [22]. The short length of *Aiptasia diaphana* nematocysts allows them to penetrate a depth ideal for transdermal drug delivery.

The *Nematostella vectensis* has spirocysts and two types of nematocysts (basitrichous haplonema and microbasic mastigophores) [23]. These nematocysts are similar to those of *Aiptasia diaphana*, but the *Nematostella vectensis* can reproduce better in a laboratory setting, making them a better option for mass production.

Transdermal Drug Delivery

Background:

In 2017, global pharmaceutical sales generated 1.143 trillion US dollars [24]. From 2006 to 2018, 66% of the mean life expectancy increase in the United States was a direct result of pharmaceutical innovation [25]. Currently, pharmaceutical drugs are delivered to humans in a variety of ways including topical, transdermal, intravenous, and most commonly, oral.

As one of the first modern drug delivery systems, oral delivery gained popularity quickly, yet in 2012, 74% of orally delivered drugs were not as effective as desired [26]. Limitations include gastrointestinal tract interaction and first pass metabolism. Hypodermic injections use the skin to deliver drugs and thus, bypass both of these challenges. This makes them a common alternative, yet injections are associated with discomfort and have a low patient compliance.

Like injections, transdermal drug delivery uses the skin to administer drugs and vaccines, but with increased patient compliance because they are less invasive, easy to apply, and self-administered [27]. In 2008, there were over a billion patches being manufactured each year and 19 approved delivery systems [28]. All of these transdermal delivery methods have to bypass the skin.

Skin and Delivery Methods:

The skin is the largest organ in the body and makes up 15% of total adult body weight [29]. Needed for physical shock protection and heat insulation, the innermost layer, known as the hypodermis is essentially a network of fat cells [30]. The middle layer, or the dermis, is 2-3mm thick and contains collagenous elastin fibers that give the skin strength and elasticity [30]. The outermost layer is the epidermis ranging from 50-100µm and is made of 5 layers [7]. With a thickness of 10-20µm, the stratum corneum (SC) is the top layer of the epidermis [27]. It consists of dead keratinocytes arranged in a dense structure [27]. The two general methods of bypassing the SC are transepidermal and transappendageal. Transepidermal methods pass either through the SC cells (for hydrophobic compounds) or between cells (for hydrophilic or small compounds) [30]. The use of hair follicles or sweat glands to transport polar compounds, ionisable compounds, and large macromolecules is transappendageal delivery [27]. Compared to transepidermal, transappendageal delivery is quite limited with delivery sites in about 0.1 percent of the total skin area [27]. Challenges like these warrant scientists to modify the SC chemically and physically by creating new transdermal delivery structures. Common transdermal drug delivery methods include patches, sprays, gels with the most effective delivery systems being physical enhancers that typically are costly reusable devices requiring electrical power [28]. There is one exception: microneedles.

Microneedles:

Microneedle technology bypasses the SC by creating miniature injections that are virtually non-invasive. Microneedles contain two types of mechanisms: those that penetrate (the microneedles) and those that support (a flat plate that holds microneedles) [31].

Microneedles come in five common types. Solid microneedles penetrate the skin and are then removed before drug application. They offer a simple approach but have skin irritation, pain, and infection risks [32, 33]. Hollow microneedles deliver drugs from a reservoir, but require extensive manufacturing resources and may clog [34]. Coated microneedles involve washing microneedles with drug formulations before skin penetration [32]. Dissolvable microneedles dissolve in the skin for controlled drug delivery, but their stability and strength are limited [32]. Hydrogel-forming microneedles absorb interstitial fluids leading to rapid swelling and drug release, facing similar challenges as other microneedles [35].

Before and during 2018, solid microneedles made up the majority of microneedle research (~35%) [36]. Dissolvable and hollow made up ~25% each and coated made up ~16% [36]. Patient delivery was mostly made up of solid and dissolvable microneedles (>30% each) [36]. As described by Tariq et al. [33], the ideal microneedle structurally should have a length between 50 to 900µm, a diameter of 1µm, withstand a force of 10N, and should not break. It should also maintain a predetermined drug delivery rate, have an increased adherence, and avoid leaks.

Results

The concept of creating non-invasive micro-injections has many possible applications. Research has suggested cnidocytes could be formulated into a gel that can deliver drugs past the SC. There has been much interest in cnidocyte compliance with hydrophilic drugs, which are usually difficult to deliver transcutaneously [37].

Oppegard et al. [20] explored Portuguese Man O' War cnidocytes, artificial discharge, puncture mechanics, and lectin binding. Physalia physalis cnidocytes were selected because they could be stored in water at 4°C longer than a year. To stimulate discharge, dried cnidocytes had to be rehydrated. Oppegard et al. [20] found after drying cnidocytes in 0.025M to 1M EDTA, an aminopolycarboxylic acid, any water-based solution would trigger discharge. Tentacle-contained cnidocytes could puncture PDMS (a silicone polymer with an elastic modulus, or MPa, of 1.00), but not Nitrile (with a MPa of 2.60). Skin has a MPa of approximately 0.13 [38]. Unfortunately, cnidocytes that were not tentacle-contained could not puncture the softest material, gelatin, because cnidocyte rotation disrupted thread discharge. One solution was binding fluorophore-conjugated lectins to the capsule surface. Con-A, a lectin, was able to bind to the cell, but failed to prevent rotation. Lectin-binding was not explored in any other key studies. This is because other studies place cnidocytes in a gel, whereas Oppegard et al [20] did not, as their goal was to incorporate them into a patch.

When choosing a cnidocyte, investigators aimed to penetrate the SC and only enter the upper epidermis [23, 37, 39, 40, 41, 42, 43]. Aiptasia diaphana cnidocytes were then chosen as they were smooth and 50μm long [41]. In 2014, Tal et al. [23] tested *Nematostella vectensis* because of straightforward cultivation and controllable sexual reproduction. When sea anemones are irritated, they release acontia, thread-like filaments full of cnidocytes. This behavior was exploited to isolate cnidocytes without harming *Aiptasia diaphana* [41].

Cnidocytes must be adapted for transdermal drug delivery. This includes changing the cationic content from Ca++ to Na+ ions because monovalent cations result in increased internal osmotic pressure (making the delivery process go faster). At this point, the cnidocytes form a powder that can be turned into an anhydrous gel.

In order to avoid unwanted drug interaction, cnidocytes could not be activated by chemicals and enzymes [41]. The gel can be spread on skin with activation from water because it can naturally carry hydrophilic drugs [41]. Similar to sea water, hydration can cause poly-y-glutamate and cations dissociation and osmotic pressure increase [37]. This allows the water-drug solution to enter the capsule and be transported into the skin after discharge. Delivery was tested using skin of a nude mouse in vitro and activated by methylene blue, a hydrophilic dye [41]. After five minutes, it was rinsed with Deuterium-depleted water (DDW) [41]. Blue coloring did not wash away in penetration spots indicating a five minute topical application completed the delivery process [41]. Administration has two steps: first, spreading cnidocyte-containing gel over skin, and second, adding water-drug solution to trigger discharge.

Alternatively, application of the water and drug can be separated, giving three steps. Using *Nematostella vectensis* nematocysts and hydrophilic lidocaine, activation and delivery were broken up. Cnidocytes were activated with water for 10 minutes, then lidocaine was applied. After four hours, delivery proved to be more effective than the control (lidocaine-only application on the skin). This method separates activation liquid and drug, bypassing the need for the drug to comply with the basic activation solution.

In a related study, the microcapsule gel was placed and activated (by saline or buffer) on 100 volunteers [37]. 30 volunteers were tested for sensitization after 10 repetitive applications [37]. No irritation or sensitization was found indicating no safety risk to this method [37]. Discharged microcapsules can be wiped off as studied in pigs [39].

Cnidocytes can deliver different organic compounds and peptides locally and systemically. There are four distinct parameters that regulate the quantity of drug delivered: the application area, the amount of cnidocytes, drug concentration, and exposure time.

Cnidocyte concentration was tested using hydrophilic lidocaine HCl [41]. It is typically seen as a non-hydrophilic topical cream for local anesthesia. Nude mouse skin testing with 5% lidocaine HCl and gel containing 0.95×10^6 cnidocytes/cm^2 was about 3 times more effective than EMLA® cream, a common numbing cream with 2.5% non-hydrophilic lidocaine, and 9 times more effective than the control gel [41].

More than 40% of the total lidocaine was delivered within 30 minutes and more than 90% within 5 hours [41]. In theory, if the amount of cnidocytes were increased, the amount of lidocaine delivered should also increase. It was found that increasing the number of microcapsules up to 10^6 microcapsules/cm^2 of skin increased lidocaine delivered [41]; however, more cnidocytes than that resulted in a decrease, likely because the microcapsules randomly oriented on the skin and prevented one another from optimally discharging.

Increasing drug concentration also increases the amount of drug delivered. This was shown by Lotan [37] when delivering salicylate and peptide using cnidocytes. Triethanolamine salicylate is found as a cream for temporary arthritic or rheumatic pain relief; however, it diffuses passively meaning it takes up to several hours for the desired effect. Testing cnidocyte delivery with 5% and 10% salicylate (by applying them for five minutes and removing them) led to delivery of 60 and 120 mg/cm^2 in 24 hours, respectively [37]. While salicylates showed a linear increase with drug concentration and drug delivery, a non-linear increase was seen with peptides. Peptides were tested with 2.5%, 5%, and 10% concentrations. The difference between 2.5% and 5% was about $20\mu g/cm^2$, while the difference between 5% and 10% was about $70\mu g/cm^2$ [37]. After 24 hours and being discharged with a 10% salicylate solution, the cnidocyte gel delivers 12 times more drug than the control gel and the control gel delivers negligible amounts of peptide [37].

In order to test effective systemic cnidocyte delivery, an *in vivo* pig study was performed. Pigs are a reliable animal model in transdermal research because their skin is highly similar to human skin with low hairiness, a thick SC, and similar dermis composition [44]. Scopolamine (a nausea medication) was the selected drug due to extensive topical delivery research in the past as it was the drug choice of the first patch. Compared to the control gel, the cnidocyte gel had peak plasma concentrations that were 5 times higher and took a significantly shorter time to reach its maximum concentration [39]. A scopolamine patch takes about 6-8 hours for the desired effect, while the cnidocyte-containing gel takes 30 minutes to reach its maximum concentration [39]. Nicotinamide and 5-Fluorouracil have also been effectively delivered through cnidocytes.

Discussion

These promising results more than a decade ago lead to an obvious question: why did cnidocyte research stop in 2017 and why have they not been implemented into modern transdermal drug delivery?

Monterey Bay Labs (formerly known as NanoCyte and StarletDerma) was a start-up company founded more than two decades ago. The company's focus was on the development of InoCyte, a system that could deliver drugs through sea anemone micro-injectors. Ayalon et al. [41] directly thanked NanoCyte in their acknowledgements and Lotan [37, 43] mentioned involvement in StarletDerma. From 2005 to 2016, Lotan and Eckhouse (the founder of Monterey Bay Lab) received 17 patents for the InoCyte design. In February of 2014, StarletDerma signed a collaboration deal with L'Oreal, a cosmetics brand. L'Oreal is the largest cosmetics brand in the world with 2022 sales equating to $37 million.

The deal outlined that InoCyte technology would be used to transdermally deliver hyaluronic acid, a cosmetic active ingredient for smoothing and filling wrinkles. No FDA approval was needed as cosmetics that make medical claims only require safety trials with 100-200 people (which were performed by Lotan) [37]. These products cannot be found online and all involvement and activity of Monterey Bay Labs ended by December, 2017. Explanations could not be found.

With hydrophilic transdermal delivery still being performed by electrically-powered devices, cnidocyte delivery should continue to be explored. Much mystery still surrounds cnidocyte classification, formation, and discharge. Better understanding these fundamental components will allow for more purposeful experiments in biotechnology.

Additionally, the cnidocyte-containing gel was only tested after being applied and activated for 5 minutes before it was wiped off the skin. Ayalon et al. [41] warrants 5 minute application by stating the system is activated immediately. By testing application times between 5 seconds and 5 minutes, it can be discovered if delivery is truly 'immediate'. Future studies should also test if hydrophilic macromolecules can be delivered using cnidocyte technology and large proteins.

There are a number of drugs that should be tested with cnidocyte delivery. One example is Nitroglycerin which is delivered orally, via infusion, or transdermally. It is used for angina pectoris, chest pain caused when the heart doesn't receive enough blood. Medication is needed short-term and long-term. In tablets, it takes 1 to 3 minutes for the desired effect which lasts up to an hour. If delivered via transdermal patch, half an hour to one hour pass before the effect is felt for eight to ten hours. Cnidocyte delivery could enable a quick initial delivery rate that slowly tapers off in the 24 hours following administration.

Conclusion

Cnidocytes are used by cnidarians for locomotion, defense, and prey capture. They also hold applications for transdermal drug delivery. Transdermal drug delivery overcomes many limitations of current oral and hypodermic methods, but has not yet reached the effectiveness of other delivery systems. More specifically, hydrophilic molecules experience difficulty in transdermal delivery. Cnidocytes overcome this obstacle, as shown in five research studies. A likely reason why their development stopped was due to a company failure and, thus, their scientific abilities

should still be explored for transdermal drug delivery in the future.

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References

1. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. Medicinal plants: Past history and future perspective. Journal of herbmed pharmacology, 2017; 7(1): 1-7.
2. Ma, Y., Zhang, Q., & Wang, H. 6G: Ubiquitously extending to the vast underwater world of the oceans. Engineering, 2022; 8: 12-17.
3. Nüchter, T., Benoit, M., Engel, U., Özbek, S., & Holstein, T. W. Nanosecond-scale kinetics of nematocyst discharge. Current Biology, 2006; 16(9): R316-R318.
4. Folino-Rorem, N. C. Phylum Cnidaria. In Thorp and Covich's Freshwater Invertebrates. Academic Press. 2015;159-179.
5. Karabulut, A., McClain, M., Rubinstein, B., Sabin, K. Z., McKinney, S. A., & Gibson, M. C. The architecture and operating mechanism of a cnidarian stinging organelle. Nature Communications, 2022; 13(1): 3494.
6. Malakhov, V. V. Symmetry and the tentacular apparatus in Cnidaria. Russian Journal of Marine Biology, 2016; 42: 287-298.
7. Lausen, B., Ahang, A., Cummins, S., & Wang, T. Investigation of Best Practices for Venom Toxin Purification in Jellyfish towards Functional Characterisation. Toxins, 2023; 15(3): 170.
8. Mackie, G. O. What's new in cnidarian biology? Canadian journal of zoology, 2002; 80(10): 1649-1653.
9. Anderson, P. A., & Bouchard, C. The regulation of cnidocyte discharge. Toxicon, 2009; 54(8): 1046-1053.
10. Özbek, S., Balasubramanian, P. G., & Holstein, T. W. Cnidocyst structure and the biomechanics of discharge. Toxicon, 2009; 54(8): 1038-1045.
11. Sugiyama, T., & Fujisawa, T. Genetic analysis of developmental mechanisms in hydra I. Sexual reproduction of Hydra magnipapillata and isolation of mutants. Development, Growth & Differentiation, 1977; 19(3): 187-200.
12. Wiebring, A., Helmholz, H., Sötje, I., Lassen, S., Prange, A., & Tiemann, H. A new method for the separation of different types of nematocysts from Scyphozoa and investigation of proteinaceous toxins utilizing laser catapulting and subsequent mass spectrometry. Marine biotechnology, 2010; 12: 308-317.
13. Kintner, A. H., Seymour, J. E., & Edwards, S. L. Variation in lethality and effects of two Australian chirodropid jellyfish venoms in fish. Toxicon, 2005; 46(6), 699-708.
14. Westfall, J. A. The differentiation of nematocysts and associated structures in the Cnidaria. Zeitschrift für Zellforschung und mikroskopische Anatomie, 1966; 75(2): 381-403.
15. Weber, J. Poly (gamma-glutamic acid) s are the major constituents of nematocysts in Hydra (Hydrozoa, Cnidaria). Journal of Biological Chemistry, 1990; 265(17): 9664-9669.
16. Cannon, Q., & Wagner, E. Comparison of discharge mechanisms of Cnidarian cnidae and Myxozoan polar capsules. Reviews in Fisheries Science, 2003;11(3): 185-219.
17. Özbek, S., Engel, U., & Engel, J. A switch in disulfide linkage during minicollagen assembly in hydra nematocysts or how to assemble a 150-bar-resistant structure. Journal of structural biology, 2002;137(1-2): 11-14.
18. Araya, J. F., Aliaga, J. A., & Araya, M. E. On the distribution of Physalia physalis (Hydrozoa: Physaliidae) in Chile. Marine Biodiversity, 2016;46(3): 731-735.
19. Munro, C., Vue, Z., Behringer, R. R., & Dunn, C. W. Morphology and development of the Portuguese man of war, Physalia physalis. Scientific reports, 2019;9(1): 15522.
20. Oppegard, S. C., Anderson, P. A., & Eddington, D. T. Puncture mechanics of cnidarian cnidocysts: a natural actuator. Journal of Biological Engineering, 2009;3(1): 1-11.
21. Singer, I. I. Tentacular and oral-disc regeneration in the sea anemone, Aiptasia diaphana: III. Autoradiographic analysis of patterns of tritiated thymidine uptake. Development, 1971; 26(2): 253-270.
22. Singer, I. I. An electron microscopic and autoradiographic study of mesogleal organization and collagen synthesis in the sea anemone Aiptasia diaphana. Cell and Tissue Research, 1974;149: 537-554.
23. Tal, Y., Ayalon, A., Sharaev, A., Kazir, Z., Brekhman, V., & Lotan, T. Continuous drug release by sea anemone Nematostella vectensis stinging microcapsules. Marine Drugs, 2014;12(2): 734-745.
24. Shamsudin, M. S., Azha, S. F., & Ismail, S. A review of diclofenac occurrences, toxicology, and potential adsorption of clay-based materials with surfactant modifier. Journal of Environmental Chemical Engineering, 2022;10(3): 107541.
25. Lichtenberg, F. R. The effect of pharmaceutical innovation on longevity: Evidence from the US and 26 high-income countries. Economics & Human Biology, 2022;46: 101124.
26. Shingade, G. M. Review on: recent trend on transdermal drug delivery system. Journal of drug delivery and therapeutics, 2012;2(1).

27. Ramadon, D., McCrudden, M. T., Courtenay, A. J., & Donnelly, R. F. Enhancement strategies for transdermal drug delivery systems: Current trends and applications. Drug delivery and translational research, 2021;1-34.
28. Prausnitz, M. R., & Langer, R. Transdermal drug delivery. Nature biotechnology, 2008;26(11): 1261-1268.
29. Kolarsick, P. A., Kolarsick, M. A., & Goodwin, C. Anatomy and physiology of the skin. Journal of the Dermatology Nurses' Association, 2011;3(4): 203-213.
30. Zaid Alkilani, A., McCrudden, M. T., & Donnelly, R. F. Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. Pharmaceutics, 2015;7(4): 438-470.
31. Zhao, Z., Chen, Y., & Shi, Y. Microneedles: A potential strategy in transdermal delivery and application in the management of psoriasis. Rsc Advances, 2020;10(24): 14040-14049.
32. Guillot, A. J., Cordeiro, A. S., Donnelly, R. F., Montesinos, M. C., Garrigues, T. M., & Melero, A. Microneedle-based delivery: An overview of current applications and trends. Pharmaceutics, 2020;12(6): 569.
33. Tariq, N., Ashraf, M. W., & Tayyaba, S. A review on solid microneedles for biomedical applications. Journal of Pharmaceutical Innovation, 2022;17(4): 1464-1483.
34. Gupta, J., Gill, H. S., Andrews, S. N., & Prausnitz, M. R. Kinetics of skin resealing after insertion of microneedles in human subjects. Journal of controlled release, 2011;154(2): 148-155.
35. Turner, J. G., White, L. R., Estrela, P., & Leese, H. S. Hydrogel-forming microneedles: current advancements and future trends. Macromolecular Bioscience, 2021; 21(2): 2000307.
36. Ingrole, R. S., Azizoglu, E., Dul, M., Birchall, J. C., Gill, H. S., & Prausnitz, M. R. Trends of microneedle technology in the scientific literature, patents, clinical trials and internet activity. Biomaterials, 2021;267: 120491.
37. Lotan, T. Immediate topical drug delivery using natural nano-injectors. Modified-release drug delivery technology, 2008;2: 395-404.
38. Diridollou, S., Patat, F., Gens, F., Vaillant, L., Black, D., Lagarde, J. M., & Berson, M. In vivo model of the mechanical properties of the human skin under suction. Skin Research and technology, 2000; 6(4): 214-221.
39. Lotan, T. Natural Nano-Injectors as a Vehicle for Novel Topical Drug Delivery. In Percutaneous Absorption. CRC Press. 2005; 557-564.
40. Lotan, T., Tal, Y., & Ayalon, A. Fast-Acting Topical Hydrophilic Drug Delivery via a Natural Nano-Injection System. Percutaneous Penetration Enhancers Physical Methods in Penetration Enhancement, 2017;343-350.
41. Ayalon, A., Shichor, I., Tal, Y., & Lotan, T. Immediate topical drug delivery by natural submicron injectors. International journal of pharmaceutics, 2011;419(1-2): 147-153.
42. Shaoul, E., Ayalon, A., Tal, Y., & Lotan, T. Transdermal delivery of scopolamine by natural submicron injectors: in-vivo study in pig. PLoS One, 2012;7(2): e31922.
43. Lotan, T. Leveraging nematocysts toward human care. The Cnidaria, Past, Present and Future: The World of Medusa and Her Sisters, 2016; 683-690.
44. Simon, G. A., & Maibach, H. I. The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations–an overview. Skin Pharmacology and Physiology, 2000; 13(5): 229-234.