A PARALLEL BETWEEN TRANSDERMAL DRUG DELIVERY AND MICROTECHNOLOGY

Florina S. ILIESCU[1], Andreea P. STERIAN (BOBEI)[2] , Marieta PETRESCU[3]

The paper built an analogy between transdermal drug delivery (TDD) and some of the micro-fabrication processes: diffusion, patterning and implantation. In this context, problems associated with TDD were presented and analyzed. We illustrated how approaches inspired by microfabrication answered to the general enquiries about the efficacy of TDD: developing a method to improve the drug-delivery through an increased diffusion coefficient. This was achieved with the help of ultrasound activating energy and of the hollow microneedles array. Diclofenac was the drug model. The experimental results showed relevant a increase in delivery efficiency.

Keywords: drug delivery, microfabrication, diffusion, transducer, sonophoresis, microneedle array, microfluidics, tissue engineering, masking layer

1. Introduction

Micro and Nano-technology started to have a huge impact on the development on new and sophisticated bio-medical devices [1-4]. Micro-total-analysis-systems (μTAS) and laboratories-on-a-chip confer advantages such as the small dimensions, easy to use, transportability and a small amount of sample required to be analyzed [5-8]. In this respect, the "hot topics" are related to (1) manipulation and characterization of little quantities of biological samples [9-11] and to (2) microfluidic approaches for tissue engineering (tissue repair and tissue regeneration) [12-16]. In the meantime, drug screening is a critical point in pharmaceutical industry where microfluidics and microfabrication can play an important role, especially in reducing the costs and the time required from drug concept to drug marketing [17].

Consequently, administration of drugs started to be a new field where micro and nano-technologies confer modern alternatives [18-19, 22]. One significant example to highlight the technological advances is transdermal drug delivery (TDD). It is generally recognized as a promising alternative to introduce medicine into the human body [18, 28] due to distinct advantages such as painless self-administration, high compliance (pediatric patients), gastrointestinal tract

[1]M.D, MSc. Republic Polytechnic, Singapore; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Lecture, University POLITEHNICA of Bucharest, Dept.of Physics, Faculty of Appl. Sciences, Romania; andreea_sterian@physics.pub.ro
[3] M.D, MSc. "Carol Davila" University of Medicine and Pharmacy, Bucharest; University Hospital Bucharest, Romania

shortcut with no first-pass effect on the administered drug, uniform diffusion of delivered compound, controlled-release and cell-targeted delivery, low cost, and reduced risk of blood-borne diseases [29-34]. Transdermal products, administered with or without incorporating various permeation enhancers [24,35-37] are already at diverse stages of formulation and clinical development with specific applications such as cardiovascular disease, Parkinson's disease, Alzheimer's disease, depression, anxiety, attention deficit hyperactivity disorder (ADHD), skin cancer, female sexual dysfunction, post-menopausal osteoporosis, and urinary incontinence. In this direction, the pharmacokinetics principles were considered: the therapeutic efficacy of a locally applied drug mainly depends on its ability to penetrate and permeate the skin [20-27].

However, the technology is still to be perfected and limitations of the TDD to be addressed [20, 21, 41-46]: (1) the low efficiency caused by the poor permeability of the SC for hydrophilic or large molecules [20, 32, 38-41] and (2) the reduced diffusion rate and little bioavailability. The present work built up an analogy between transdermal drug delivery (TDD) and some of the microfabrication processes: diffusion, patterning or ion implantation. Diclofenac was considered as a drug model (as diffusant source) on different enhancing methods using: bulk microneedles array, hollow microneedles array, sonophoresis and sonophoresis coupled with the hollow microneedle array. These methods were compared with the passive diffusion of the drug. We noticed that the simple "micropatterning" of the Stratum Corneum using bulk microneedles array increased the diffusion of the drug with almost one order. Using hollow microneedles, a sensitive increment of the drug diffusion can be achieved by moving the diffusion point inside the skin. Lastly, further increment can be achieved increasing the "activation energy" of the drug (similar with the classical microtechnological process of ionic implantation) by merging the sonophoresis with diffusion through hollow microneedles. As a result, microfabrication processes can be valuable inspiring sources for improving the efficiency of TDD.

2. Materials and methods

2.1 Skin model preparation

Histologically and physiologically, pig skin is the closest to the human skin. Therefore, we prepared the in-vitro test on pig skin to demonstrate the drug delivery with the microneedles array. The fresh porcine skin sample collected from the rear abdomen was prepared in compliance with relevant regulations approved by the Institutional Animal Care and Use Committee of National University of Singapore and then stored in aluminum foil, at –80ºC. Fig. 1 depicts the skin sample after preparation and before storing it.

The steps in skin preparation protocol were:

1. Excise the skin from abdominal area of pigs;
2. Remove the hair from the skin sample;
3. Remove the adhering fat and other visceral debris by tweezers;
4. Scrape off the underlying subcutaneous fat to leave the skin to be 1 to 1.5 mm thick;
5. Wash the skin with physiological saline;
6. Wrap the skin in aluminium foil;
7. Store at -20^{o}C

Fig.1: Freshly excised pig skin specimen of 1 mm thick (the epidermis layer faces up after subcutaneous tissues were carefully removed)

2.2 Materials for testing

The drug model tested was Diclofenac (296.148 g/mol) as sodium salt (75 mg diclofenac sodium in 3 ml aqueous injectable solution). The permeability and transport of Diclofenac across the skin were detected using UV-visible spectrophotometry (Agilent 8453 UV– vis system). The excitation wavelength was 280nm.

2.3 Transdermal delivery device

We considered different systems for increasing the efficiency of drug delivery:

- bulk microneedles that were used to generate micro-perforations in the SC (temporary applied for few minutes before drug testing)
- hollow microneedles array with inner hydrophilic holes
- low frequency sonophoresis (using a PZT transducer)
- combination of hollow needles with low frequency sonophoresis

All these methods were reported to increase the diffusion through the skin.

Figure 2a presented the bulk microneedles array used in the experiment. The bulk microneedles were fabricated on silicon wafer using standard microfabrication processes described in[47-49]. The microneedles were pyramidal shape of 100μm-high.

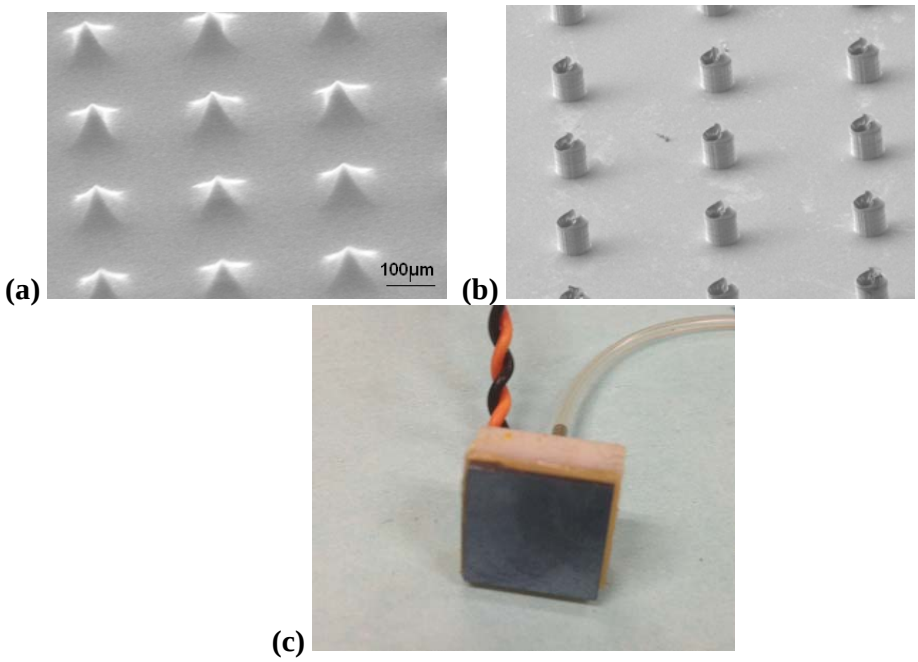

Fig. 2: Images of (a) the bulk microneedles patch, (b) the hollow microneedles patch and (c) the device employing sonophoretic delivery through hollow microneedles

The geometrical features of the hollow microneedles used were: 100μm in length, 80μm the outer diameter at the base, and 30×30 matrix on a 12mm×12mm silicon chip. The penetration length, preferable of less than 100μm, could be justified by the depth of stratum corneum (5–20μm) and by variable depth of the epidermis (20–100μm). The inner-holes can be designed to be eccentric to the outer-ring, in order to generate the slanted tip of the microneedles and facilitate penetration into the skin. Both the size and the density of the microneedle array can play an important role in TDD's efficacy. Therefore, the geometry is to be considered carefully when designing the microneedles array. The hollow microneedles were processed by microfabrication technologies described in [50].

A miniaturize device consisting of a chip with hollow microneedles, a PZT(lead zirconate titanate) transducer packaged in a plastic housing fabricated using polijet printing is presented in Fig. 2c. The ultrasonic emitter was set at 20 kHz, 20% duty, 0.1~1 W/cm^2.

2.4 In vitro drug delivery test's setup

We used a setup, as shown in Figure 3, comprising a Franz diffusion cell (Logan Instruments Corp., Somerset, NJ) and conducted the in-vitro drug release test for Diclofenac.

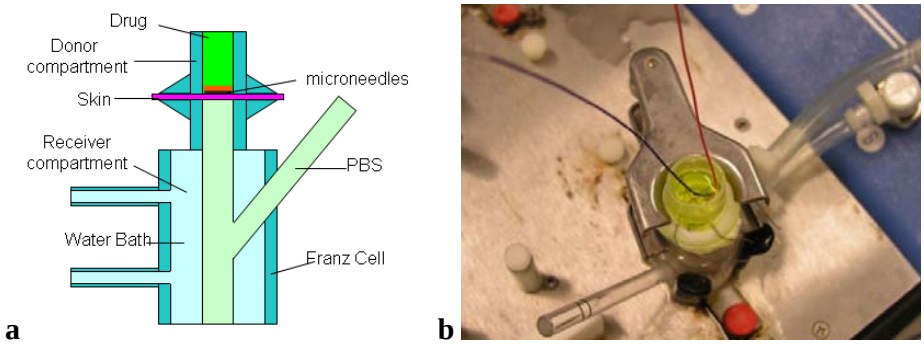

Fig. 3: Illustration of the *in vitro* transdermal delivery with the hollow microneedles enhancer (a) and using sonophoresis (b)

The diffusion area was of 1.4cm^2 and the receptor compartment volume of 15cm3(15ml). The solutions, drug model, phosphate-buffered saline (PBS) solution and the water bath, were thermo-stated at 37^{o}C. The prepared porcine skin sample was removed from the fridge one hour prior each test start and mounted on to a Franz diffusion cell, with the stratum corneum side facing to the donor compartment. The microneedle array was then pressed onto the stratum corneum, and held by the donor compartment. The donor and receiver compartments were subsequently clamped together. The receiver compartment was filled with phosphate buffered saline (PBS) solution while the temperature of water bath was maintained at 37°C. We conducted four parallel sessions, which tested the transport of the drug across the skin into the receiver compartment using microneedles (bulk and hollow) array and the passive diffusion using the control group.

3. Results and discussion

We used the porcine skin as the substrate for the microneedles insertion to generate conduits or microchannels for the transport of drugs across the stratum corneum. Through these microchannels, drug diffused rapidly towards the deeper layers of skin, implicitly the capillaries.

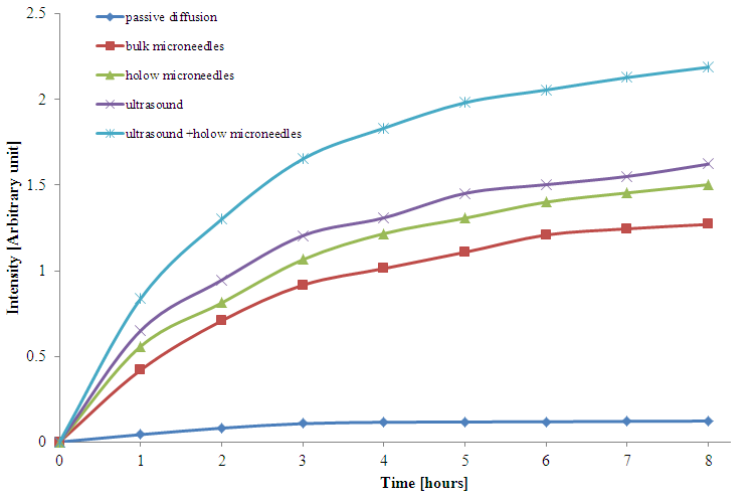

Figure 4: The drug release profiles for simple diffusion, bulk microneedles facilitated diffusion, hollow microneedles facilitated diffusion, sonophoresis facilitated diffusion and ultrasound plus hollow microneedles facilitated diffusion

The drug release profiles of the five types of transdermal delivery, simple diffusion, bulk microneedles facilitated diffusion, hollow microneedles facilitated diffusion, sonophoresis facilitated diffusion and ultrasound plus hollow microneedles facilitated diffusion, were compared and the results are shown in Fig. 4. The skin permeability for Diclofenac was greatly increased once an enhancing method was used: approximately 10 times for bulk microneedles, up to 20 times for the method that combined sonophoretic delivery with hollow microneedles.

It is known that the transdermal drug delivery mechanism is the classical process of diffusion through which molecules are transported from the diffusion source to the target. If we consider the case of market-available patches, the medication is delivered across the stratum corneum, the external layer of the skin, towards the deeper structures and eventually the systemic circulation. Stratum Corneum is a hard layer of 10-20μm thickness, with low permeability and with a robust structure containing dead cells embedded in a continuous structure of lipid bilayers. If it is compared with microtechnology processes, it can be considered as a “masking layer” for the drug diffusion. An illustration of this aspect is presented in Fig. 5.

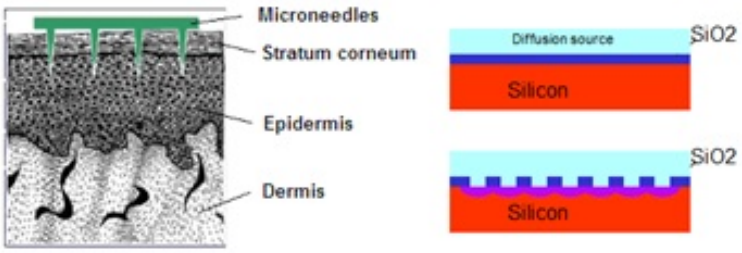

Fig. 5: a schematic representation of the s skin's histology and its equivalent from the microfabrication perspectives

Moreover, the drug flux F through the skin is proportional with the concentration gradient as given by the Fick's first law:

$$F = -D\frac{\partial C}{\partial x} \tag{1}$$

where D is the diffusion coefficient while $\partial C/\partial x$ is the concentration gradient. The flux gradient $\partial F/\partial x$ is proportional with the change of concentration in time and is approximated by the Fick's second law of diffusion:

$$\frac{\partial C(x,t)}{\partial t} = -\frac{\partial F}{\partial x} = D\frac{\partial^2 C}{\partial x^2} \tag{2}$$

where the concentration C is a function of position x and time t, while D is assumed to be constant. According to the above mentioned conditions, eqn. can be simplified to:

$$\frac{dm}{dt} = D\frac{C_0}{h} \tag{3}$$

and, subsequently

$$D = f(Ea, T). \tag{4}$$

where m is the mass of permeant that passes per unit area through the membrane in the time t, Co is the concentration of the source and h is the membrane thickness, D is the diffusion coefficient, Ea is the activating energy and T is the temperature.

In our approach, we firstly considered the fact that the essential target of the entire work on microneedles is to ensure a noninvasive transmission of the drug molecule across the stratum corneum. Therefore, we discussed the design and fabrication from two perspectives: (1) the skin model and (2) Fick's first law.

The histological considerations evaluated the microneedles' interaction with the SC and the ability of microneedles to shortcut this superficial layer of the skin. From the physics perspective, we considered the diffusion coefficient D as a function of the activating energy Ea and temperature. Fig. 5 depicted the skin equivalent in microneedles' microfabrication and Equation 1 explained the

relationship between m the mass of permeant that passes per unit area through membrane in the time t, and the C_o the concentration of the source and h the membrane thickness. One clinical application of Equation 4 could be discussed here: the impossibility to perceptively modify the temperature which implies the need of increasing the activating energy of the diffused molecules by other means. Therefore, we proposed low frequency ultrasound as diffusion enhancer.

The pharmacological and clinical approaches consider possible ways for drug to be transported based on chemical and physical principles. Since drug permeation across the stratum corneum obeys Fick's first law, a drug molecule must follow it to meet the physicochemical properties as in case of nicotine or nitroglycerin. As the majority of the therapeutic agents do not fulfil these criteria, the need of methods to enhance diffusion apart of chemical means[37] was discussed extensively. Therefore, physical enhancers were introduced to help molecules by pass the stratum corneum either by removing it [51] or by using mechanical [46, 52], electrical [53,54], thermal [55] or ultrasonic energy [56-58]. Mechanical means of TDD employed biodegradable materials [59-61] to increase the efficiency level of the procedure. Permeation enhancers could facilitate the drug delivery across the skin when used in combinations. Therefore, we considered sonophoresis coupled with hollow microneedles as one modality to study how permeation could further be improved for a therapeutic agent, Diclofenac. We based the experiment on the principles of microfabrication of TDD.

4. Conclusions

The tests' results indicated the great enhance in the skin permeability for a better drug transport across the skin. Since we compared the simple diffusion with mechanically enhanced TTD and sonophoresis, the data obtained showed that the microchannels and pathways in the skin tissues created by the microneedles, and by the concomitantly applied low-frequency-ultrasound were responsible for the magnified transportation across the skin. The results indicated the real potential of microneedles coupled with sonophoresis as a more effective transdermal drug delivery system with significant clinical application.

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