Application of Tixel for Transdermal Delivery

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ABSTRACT

**Background:** Tixel is a novel device for thermal fractional skin treatments with low pain in ablative and non-ablative modes.

**Objective:** This study is aimed to evaluate the drug permeation properties of skin treated with the Tixel system in non-ablative mode.

**Methods:** Tixel S tip at 400°C was applied on skin for 9ms. Skin permeability was evaluated by in vivo confocal microscopy for visualization of the human skin channels as well as by a diffusion cell system using excised porcine ear skin. Verapamil hydrochloride 1% was used as a hydrophilic model drug known to have a poor absorption across mammal skin.

**Results:** Decomposition of the stratum corneum after Tixel treatment was visualized by confocal microscopy. A matrix of open channels is created painlessly without bleeding in the epidermis allowing diffusion of topical fluoresceine within 2 minutes down to the upper dermis in channels that have been formed 6 hours earlier by Tixel. At the same parameters, skin permeability to Verapamil was measured and compared to untreated skin. After 24 hours, the cumulative amount of drug that had penetrated across the skin was 20 fold higher than across the untreated skins.

**Conclusions:** Fractional Tixel effectively opens the skin for transdermal delivery of compounds with no pain, no bleeding and no downtime.

**Keywords:** verapamil, transdermal delivery, permeation, Franz cell, fluorescent
Introduction

Transdermal delivery constitutes a large market for both aesthetic and medical applications. The skin is a highly impermeable barrier that allows only small lipophilic compounds to penetrate into it (1). Disruption of the skin outer layer, the stratum corneum, increases its permeability. A number of approaches have been developed in order to disrupt the stratum corneum such as mechanical, chemical or physical (2, 3, 4). One successful method to increase permeability is by heat. Prausnitz et al showed in references 5 and 6 that short exposure to high temperature dramatically increases skin permeability and that thermal damage can be localized to the stratum corneum without damaging deeper tissue. A strong positive correlation between temperature and skin permeability was found. Thermal ablation of skin that selectively ablated the Stratum Corneum, dramatically increased skin permeability for transdermal drug delivery. Above 360°C, transdermal flux increased by 3 orders of magnitude, associated with decomposition and vaporization of keratin to create micron-scale holes. Finally the authors claimed that a hundred micron diameter pores should allow passage of a wide range of hydrophilic and macromolecular drugs although at different diffusion rates.

The Tixel fractional treatment device is a novel thermal resurfacing system which can generate ablative as well as non-ablative micropores in the skin (8,9). Superheating water molecules in the skin tissues with a high conductivity metallic tip is effective in damaging the skin cells in a safe, precise and predictable manner. Tixel’s D tip creates ablative micropores with similar properties to fractional CO₂ lasers. Tixel’s S tip can generate non-ablative "dressed" micropores with underlying thermal damage extending down to the papillary dermis.

This study focuses on thermal decomposition of the stratum corneum using Tixel to increase skin permeability by heat. The channels formed in the skin have been evaluated by histology and by in vivo confocal microscopy. Permeability of the skin was tested with a small fluorescent dye using confocal imaging as well as by diffusion cell using a hydrophilic molecular model, Verapamil Hydroxide with 491.06Da.
Methods

2.1 The Tixel

The Tixel (Novoxel, Israel) is a thermo-mechanical system for fractional ablation. It applies a tip, which is made of metallic, gold plated biocompatible materials (Fig. 1). The tip is fixated at the distal section of the Tixel handpiece which is equipped with a linear motor (Fig. 1). The tip’s active surface consists of an array of 81 (9x9) pyramids evenly spaced within a boundary area of 1x1cm. The pyramids are 1.25 mm tall having a radius of about 100 microns at the edge. The back plane of the tip is flat and is connected to a coin-size heater which is kept at a constant temperature of 400°C during operation. When not in use, the tip is base-positioned at a distance of 2cm from the skin’s surface (home). The tip weighs 7 grams and is re-usable. The system checks, validates, cleans, sterilizes and exchanges tips automatically. Tip cleaning after treatment is performed within 5 minutes at 520°C, while the tip is still mounted on the handpiece and tip automatic self-sterilization is performed within 3 minutes at 350°C. Tip cleaning, tip sterilization and tip biocompatibility have all been validated. The handpiece weighs 270 grams. Two tip types are available: D with high thermal conductivity and S with low thermal conductivity. When the user activates the handpiece, the linear motor rapidly advances the tip which comes in brief contact with the tissue. Thermal energy is transferred to the skin, creating micropores in it by evaporation. The tip recedes within a precisely controlled distance and time to its home position, away from the tissue. The duration of the pulse, i.e. time of contact between tip and skin, can range from 6ms to 18ms. A double pulsing mode is also enabled. The motor’s displacement accuracy is in the range of 1-8 μm. A 14ms pulse delivers a high energy pulse of ~25 mJ/pore while a 10 ms pulse delivers a medium energy pulse of ~15 mJ/pore and a 6ms pulse delivers a low energy pulse of ~10 mJ/pore. The theoretical and engineering foundations of the Tixel technology have previously been described in Lask et al, 2012 (7). The utilization of the Tixel does not require the use of protective eyewear or a smoke evacuator.

2.2 In vivo confocal microscopy of the human Tixel channel

Confocal imaging was performed using a fluorescent confocal microscope (Vivascope 1500 multilaser, Lucid Inc, Rochester, USA) on 6 volunteer’s arm. A fluorescent probe, fluorescein SE thilo, was placed for 2 minutes on skin areas previously treated with Tixel (parameters: S Tip, 9ms single pulses) 2 and 6 hours before and then sponged out. Permeation of fluorescence up to 200 microns deep in the skin layers was visualized.
2.3 Transdermal delivery of Verapamil on ex vivo pig skin by Franz cell

Full-thickness porcine skins were excised from fresh ears of white pigs (Kibbutz Lahav, Israel). Only pieces that the TEWL levels were less than 10 g/m²/h were used. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee.

Excised skins were treated or untreated with Tixel (Tixel, NOVOXEL ltd, Israel). A S-Tip was operated at 400°C for 6ms or 9ms.

Immediately after skin treatment, the permeability of Verapamil hydrochloride 1\% (Euroasian Chemicals Ltd., Mumbai, India; MfD: 02/2014) through porcine skin was determined in vitro with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ) [9]. The diffusion area was 1.767 cm² (15 mm diameter orifice), and the receptor compartment volume was 12 ml. The solutions in the water-jacketed cells were thermostated at 37°C and stirred. Samples (2 ml) were withdrawn from the receiver solution at predetermined time intervals (3, 5, 7, 9, 12, 20 and 24 hours) and kept at -20°C until analyzed by HPLC. Aliquots of 20 µl from each vial were injected into HPLC system (Shimadzu VP series including diode-array detector for peak spectrum identification), equipped with a pre-packed C₁₈ column (Betasil C18, 5µm, 250X4.6mm, ThermoHypersil, UK) heated to a temperature of 30°C. Data were expressed as the permeating drug quantity per unit of the skin surface area, $Q_t/S$ ($S = 1.767 \text{ cm}^2$). Cumulative drug permeations ($Q_t$) were calculated as in [10].

The statistical differences were analyzed by the two-way unweighted means analysis of variance (ANOVA) test. The differences among group means were considered significant for $p$ values < 0.05. Results are given as average ± standard deviation.

**Histology**

Treated skin samples were fixed in formalin, embedded in paraffin and stained for H&E for histopathological examination.
RESULTS

Tixel’s skin channel characterization

As shown in figure 2, tiny lesions are created in the skin by Tixel with the S tip at 9ms pulse duration affecting the stratum corneum and epidermis. In some cases, the epidermis stays intact. There is minimal damage to the dermis with minimal dermal coagulation. In vivo treatments were without pain, erythema disappeared within a day on facial treatment sites.

Visualization of the channels by confocal microscopy shows a clean hole in the epidermis down to the upper dermis (figure 3). Figure 4 shows stratum corneum decomposition.

Transdermal Delivery of fluorescein

The passage of a small fluorescent hydrophilic molecule through channels created 2 and 6 hours previously, at the same Tixel settings, on the in vivo human arm was tested by confocal microscopy. The dye permeated through the stratum corneum barrier and diffused into the epidermis and upper dermis in both cases (figure 5).

Transdermal Delivery of Verapamil

The penetration of verapamil hydrochloride increased about 5 and 20 times after skin had been pretreated with Tixel TMA system for 6 ms and 9 ms, respectively (Q_{24} = 168.16± 93 µg/cm² (n=8) and 728 ±358 µg/cm² (n=11) compared to Q_{24}=33.75 ± 25.3 µg/cm² (n=5) in untreated skin. The statistical tests revealed highly significant differences between the experimental groups (p<0.05) (Fig 6). In addition, lag time to get quantitative permeation across the skin decreased significantly from 9-10 hours in untreated skin to 3-5 hours in Tixel-treated skin.

DISCUSSION AND CONCLUSIONS

Confocal microscopy confirmed that the Tixel, at low energy is able to disrupt the stratum corneum and form channels in the skin that stay open for the diffusion of a fluorescent dye for up to 6 hours post Tixel application, which could be useful for drug delivery and combination treatment with cosmetic products. Treatment is painless with minimal crusting. Erythema clears within 24 hours.
REFERENCES


FIGURES and LEGENDS

**Figure 1:** The Tixel handpiece and its metallic tip
Figure 2: Skin histologies immediately after Tixel treatment (S tip, 9 ms pulse). (A, B, C) - in vivo treatment of a male 61 years. (D & E) – ex vivo treatment of a freshly excised human skin. (F) in vivo treatment of a 5 weeks pig. (G) ex vivo treatment of pig’s ear.
**Figure 3:** Decomposition of the stratum corneum (red circles) as viewed by in vivo confocal microscopy on human skin. Treatment setting: S tip, 9ms pulse.
Figure 4: Clean channel visualized by in vivo confocal microscope on human skin. Red circle shows the channel (S tip, 9ms pulse). Red circle shows the channel. (A) skin surface. (B) Epidermis layer. (C, D) Upper dermis layer.
**Figure 5:** In vivo fluorescent confocal microscopy of Tixel (S Tip, 9ms single pulse) channel on human skin. Diffusion of the fluorescent compound is visualized through the epidermis and upper dermis. Upper row - Tixel was applied on the skin 2 hours prior to imaging, (A) 36μm deep, (B) 54μm deep, (C) 101μm deep. Lower row - Tixel was applied on the skin 6 hours prior to imaging, (D) 30μm deep, (E) 67μm deep, (F) 113μm deep.
Figure 6: Verapamil delivery through Tixel pretreated skin at 6ms and 9ms pulse and untreated skin. The penetration of verapamil hydrochloride increased about 5 and 20 times after skin had been pretreated with Tixel TMA system for 6 ms and 9ms, respectively (Q_{24} = 168.16\pm 93 \, \mu g/cm^2 \, (n=8) and 728 \pm 358 \, \mu g/cm^2 \, (n=11) compared to Q_{24}=33.75 \pm 25.3 \, \mu g/cm^2 \, (n=5) in untreated skin. The statistical tests revealed highly significant differences between the experimental groups (p<0.05). In addition, lag time to get quantitative permeation across the skin decreased significantly from 9-10 hours in untreated skin to 3-5 hours in Tixel-treated skin.

Verapamil delivery through Tixel pretreated skin

![Cumulative drug permeation over time](image-url)