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Research paper

Microneedle-assisted delivery of verapamil hydrochloride and amlodipine besylate

Monika Kaur [a], Kevin B. Ita [a,*], Inna E. Popova [b,1], Sanjai J. Parikh [b], Daniel A. Bair [b]

[a] College of Pharmacy, Touro University, Mare Island-Vallejo, CA, USA
[b] Department of Land, Air and Water Resources, University of California, Davis, CA, USA

ABSTRACT

The aim of this project was to study the effect of stainless steel solid microneedles and microneedle rollers on percutaneous penetration of verapamil hydrochloride and amlodipine besylate.

Verapamil, 2-(3,4-dimethooxyphenyl)-5-[2-(3,4 dimethoxyphenyl)ethyl-methyl-amino]-2-propan-2-yl-pentanenitrile is a calcium channel blocker agent that regulates high blood pressure by decreasing myocardial contractilty, heart rate and impulse conduction. Amlodipine, (R, S)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1, 4-dihydropyridine, is a calcium channel blocker that is used for the management of hypertension and ischemic heart disease. Passive penetration of verapamil and amlodipine across the skin is low. In vitro studies were performed with microneedle-treated porcine ear skin using vertical static Franz diffusion cells (PermeGear, Hellertown, PA, USA). The receiver chamber contained 5 ml of PBS (pH7.4) and was constantly maintained at 37 °C temperature with a water circulation jacket. The diffusion area of the skin was 1.77 cm². The donor compartment was loaded with 1 ml of the solution containing 2.5 mg/ml of amlodipine besylate. The donor chamber was covered with parafilm to avoid evaporation. Passive diffusion across untreated porcine skin served as control. Aliquots were taken every 2 h for 12 h and analyzed by liquid chromatography–mass spectrometry. Transcutaneous flux of verapamil increased significantly from 8.75 μg/cm²/h to 49.96 μg/cm²/h across microneedle-roller treated porcine skin. Percutaneous flux of amlodipine besylate following the use of stainless steel microneedles was 22.39 μg/cm²/h. Passive flux for the drug was 1.57 μg/cm²/h. This enhancement of amlodipine flux was statistically significant. Transdermal flux of amlodipine with microneedle roller was 1.05 μg/cm²/h in comparison with passive diffusion flux of 0.19 μg/cm²/h. The difference in flux values was also statistically significant. Stainless steel solid microneedles and microneedle rollers increased percutaneous penetration of verapamil hydrochloride and amlodipine besylate. It may be feasible to develop transdermal microneedle patches for these drugs.

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1. Introduction

According to the American Heart Association about 76.4 million people over age of 20 and older have high blood pressure in the United States [1]. According to the World Health organization, suboptimal BP (>115 mmHg SBP) is responsible for 62% of cerebrovascular disease and 49% of ischemic heart disease [2]. Environmental factors or genetics usually predispose individuals to hypertension. High sodium intake, lack of exercise, stress levels or obesity can result in high blood pressure. Essential hypertension can go undiagnosed as it does not have identifiable cause. Secondary hypertension is usually caused by another condition such as kidney disease or tumors [3].

Several approaches have been used to manage hypertension. Angiotensin converting enzyme (ACE) inhibitors are used to lower the vasoconstrictor angiotensin II. Angiotensin receptor blockers act on AT1 and AT2 receptors to interrupt the action of angiotensin II [3]. Calcium channel blockers (CCBs) are antihypertensive agents used to mediate the transport of calcium in cardiac and vascular cells. CCBs help relax blood vessels to reduce the workload of the heart.

In this study, two antihypertensive drugs, verapamil and amlodipine, were investigated for transdermal drug delivery. Verapamil, 2-(3, 4-dimethooxyphenyl)-5-[2-(3,4 dimethoxyphenyl) ethyl-methyl-amino]-2-propan-2-yl-pentanenitrile, is a calcium channel blocking agent. It is used in treatment of hypertension, angina pectoris and supraventricular arrhythmias [4]. Increased calcium channel blockers concentration in the plasma would result

improved cardiovascular condition. Transdermal delivery of verapamil will increase the concentration of the systemic verapamil that would increase therapeutic efficacy. Amlodipine, (R, S)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1, 4-dihydropyridine, is also a calcium channel blocker that is used to treat hypertension and ischemic heart disease [5].

Amlodipine partition coefficient in two different phases was reported in the literature. The two phases were n-octanol/water and n-octanol/phosphate buffer saline. Log K values obtained were 2.96 and 3.09, respectively [6]. Verapamil partition coefficient (log P) value obtained using octanol and water was 3.8 [7]. The permeability of these drugs across the skin is low. Several strategies have been used to overcome the excellent barrier properties of the stratum corneum to enhance transdermal drug delivery. One approach is the application of microneedles to increase percutaneous absorption.

Transdermal microneedles create micron sized pores in the skin to enhance delivery of the drug across the skin. Micron sized needles are ideal for patient adherence as they do not stimulate nerves that are associated with pain. Microneedles improve patient compliance as patient with needle phobia will be more likely to apply the patch because of its painlessness. Additionally, patients can administer the drug by themselves [8]. Microneedle-mediated drug delivery only disrupts the stratum corneum and epidermis but it does not reach nerve fibers and blood vessels in the dermis [9]. Patients with gastrointestinal tract irritation are more likely to benefit from the use of microneedle mediated patches as the drug gets directly into systemic circulation. Furthermore, drugs with poor absorption rate and instability are ideal for transdermal drug delivery [8]. Transdermal drug delivery is beneficial to patients with chronic conditions such as diabetes. Furthermore, it is useful for patients that have poor adherence as they are taking multiple medications or going through illness where they simply forget to take the medication at the right time such as those suffering from Alzheimer's disease. Microneedle-mediated transdermal drug delivery reduces skin irritation [10].

Factors that affect the diffusion rate across the skin are microneedle density, microneedle length, number of applications and drug concentrations. According to Gomaa et al., increase in the microneedle density led to increase in the level of drug penetration across the skin [11]. The studies also demonstrated that application of longer microneedles increased the drug diffusion through skin. Multiple applications of the microneedles and higher concentration of the drug solution in the donor chamber of the Franz vertical cells led to an increase in transdermal drug delivery.

Hollow microneedles are designed with channels that deliver medications and extract biological fluids [9]. Controlled drug diffusion is achievable with hollow microneedles where drug delivery rate can range from fast to slow diffusion and it can be used for time-dependent delivery across the skin. Hollow microneedles can be fabricated from glass, polymer and metal. Insulin delivery through hollow microneedles was shown to reduce the blood glucose levels in type 1 diabetic patients [8]. During hollow microneedle application, upper layer of the skin is disrupted and the drug is released into the skin. This is known as 'poke and flow' [12]. These microneedles are used to deliver liquid drugs across the skin as it is facilitated by pressure-driven flow [8].

Solid microneedles are applied to skin with pressure that creates micron sized holes in the skin for penetration of the drug across the skin [8]. Silicon, polymers, metal and ceramic are examples solid microneedles. Metals such as stainless steel have been used to create needles (i.e. hypodermic needles) for decades [9]. Furthermore, stainless steel microneedles provide good mechanical strength and are easy to cut with laser beam [13]. Microchannels are created after insertion and removal of the microneedles,

and then drug loaded patch is applied for the penetration of the drug across the skin. This mechanism is known as 'poke and patch'. Solid microneedles create microchannels that can remain open for period of 72 h under occlusive conditions [12]. In vivo studies have been conducted that showed that solid stainless steel microneedle application followed by insulin patch lowered blood glucose levels by 80% in rats [14].

Microneedle rollers (dermarollers) are stainless steel microneedle-embedded cylindrical surface that rolls over the skin to create micropores. Badran et al. studied the effect on microneedle length and chemical penetration enhancers on the transdermal penetration of hydrophilic drug molecules across full thickness human skin. The hydrophilic substances investigated in the experiment were carboxyfluorescein and radiolabeled mannitrol. Micropores created by dermarollers were visualized using light microscopy and scanning electron microscopy (SEM). Skin treated with dermaroller had higher transepidermal water loss values in comparison with intact skin [15].

The aim of the present study was to investigate microneedle-mediated transdermal delivery of antihypertensive agents – verapamil hydrochloride and amlodipine besylate across pig skin. Stainless steel microneedles and microneedle rollers were used in our investigation to determine transcutaneous fluxes of verapamil and amlodipine across pig skin. This paper focuses on microneedle-assisted transdermal delivery of verapamil hydrochloride and amlodipine besylate through porcine ear skin.

2. Materials and methods

2.1. Chemicals and reagents

Phosphate buffered saline (PBS), verapamil hydrochloride and amlodipine besylate were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Porcine ears were obtained from Animal Technologies (Tyler, TX, USA). Double distilled ionized water was prepared using NanoPure Infinity Ultrapure water purification system.

2.2. Stainless steel microneedles

Adminpatch stainless steel microneedle arrays (manufactured by NanoBioSciences LLC, Sunnyvale, CA) were supplied by Stanford University Biomaterials and Advanced Drug Delivery Laboratory. These were used to create microchannels in porcine skin for transdermal drug delivery. Microneedle rollers were purchased from New Skin Care Collection, Tallahassee, Florida, USA.

2.3. Skin preparation

Institutional Animal Care and Use Committee (IACUC), Touro University, Mare Island-Vallejo, CA approved the experiments. Pig ears were obtained from Animal Technologies (Tyler, Texas, USA). Skin pieces were thawed at room temperature and cleaned with tap water. An electric clipper was used to trim pig ear hairs. An electric dermatome (Robbin's Instruments, Chatham, NJ, USA) was used to section the skin to a thickness of 550 µm. Porcine ears were then stored at a temperature of −20 °C for no longer than two months before use.

2.4. Diffusion studies

In vitro permeation experiments were conducted using vertical Franz diffusion cells. Microneedle-treated porcine skin, dermatomed to 550 µm was mounted on the donor chambers of three cells having an area of 1.77 cm^2. Three cells with untreated pig skin served as controls. The volume of the diffusion cells was 12 ml. The

upper donor chamber and lower receiver chamber of the Franz vertical cell were fastened together by means of a clamp, along with skin between the two chambers. 2 ml of verapamil solution (50 mg/ml) or amlodipine besylate (2.5 mg/ml) was loaded onto donor chambers and covered with Parafilm. Aliquots of the receptor solution were collected at the interval of 2 h for a 12 h period. The samples were stored at 4 °C until analysis by liquid chromatography–mass spectrometry (LC–MS). Receptor chambers were replenished by an equal volume of fresh phosphate buffer solution. All of the six receptor chambers contained a magnetic stirrer to maintain constant concentration.

2.5. LC–MS analysis

HPLC/DAD-ESI/MS/MS analysis was performed using an Agilent series 1200 HPLC with diode-array and Agilent 6320 Ion Trap mass spectrometer detector (Agilent Technologies, Palo Alto, CA). Chromatographic separation was carried out on the reverse-phase Agilent Zorbax Eclipse Plus C18 (100×2.1 mm, 3.5 μm) analytical column, which was protected by a guard column with the same stationary phase (12.5×4.6 mm, 5 μm) (Agilent Technologies, Palo Alto, CA). Column temperature was set at 40 °C, and autosampler temperature was set at 4 °C. The mobile phase consisted of 0.1% formic acid in water (solvent A), and 0.1% formic acid in methanol (solvent B). The gradient was performed at 0.4 ml/min with an initial condition of 10% of mobile phase B for 2 min. Mobile phase B was increased to 90% at 3 min and held at 90% B to 8 min. Calibration curve standards were freshly prepared in PBS buffer solution. The MS data were collected in the positive ESI MS/MS mode. Nebulizer temperature was 350 °C, nebulizer pressure was 50 psi, and the drying gas flow rate was 10.0 l/min. Compounds were quantified in positive ESI MS/MS mode by quantifying specific parent ion. UV spectra were collected for each compound.

2.6. Characterization of the microneedles and microconduits – SEM

Scanning electron microscopy (SEM) was performed at the Electron Microscopy Laboratory, Department of Medical Pathology and Laboratory Medicine, School of Medicine, University of California at Davis. SEM was used to visualize stainless steel microneedles and the microchannels created in porcine skin. The device was mounted onto an aluminum stub using DUCO cement mix with carbon shavings (for conductivity), sputter coated with gold using a PELCO SC-7 coater and viewed on the FEI XL 30 scanning electron microscope. Skin was fixed with Karnovsky's fixative in 0.1 M sodium phosphate buffer then washed with 0.1 M sodium phosphate. Dehydration was accomplished in increasing concentrations of ethanol through 100% ethanol and critical point dried in a Tousimis 931.GL Autosamdri critical point dryer. The mites were mounted on aluminum stubs and sputter coated with gold using a PELCO SC-7 coater. The samples were viewed on an FEI XL30 TMP SEM.

2.7. Statistical analysis

Sigmastat software was used for statistical analysis. Mann–Whitney rank sum test was used to determine the statistical significance of data obtained from the experiments. Mean of replicate measurements ($n = 3$) with corresponding standard errors (SE) was used to plot the graphs.

3. Results

3.1. Characterization of microneedles and microconduits

Stainless steel microneedles were applied onto porcine skin to study percutaneous penetration of antihypertensive drugs –

verapamil hydrochloride and amlodipine besylate. The microneedles had a density 187 needles/cm^2 and each microneedle was 500 μm long.

DermaRoller is a device that contains microneedles mounted on cylindrical surface that rolls over the skin. There were 540 microneedles mounted on cylindrical drum that were 500 μm in length. The images (Figs. 1a–1c) were collected using HTC one-X camera.

Scanning electron microscopy was used to visualize the stainless steel microneedles and microconduits. The top view shows that microneedles were aligned symmetrically. The image from the side view shows that needles are sharp-tipped. SEM image of single microneedle determines the length and width of the microneedle (Figs. 2a–2c).

Microconduits created across pig skin by microneedles determine the disruption of the stratum corneum, which acts as an excellent barrier to transdermal penetration. It also shows that microneedles successfully penetrated the skin to create micropores. Fig. 3a demonstrates that microchannels are symmetrical across porcine skin. Single microconduit has diameter of 100 μm that allows diffusion of small hydrophilic molecules (Fig. 3b).

3.2. In vitro permeation studies

In vitro permeation experiments were conducted to study the transdermal delivery of two antihypertensive agents – verapamil hydrochloride and amlodipine besylate. The experiments were performed on porcine skin using stainless steel microneedles. Porcine skin was used to investigate transdermal drug delivery because it is a good model of human skin. Pig ear skin was mounted between donor and receiver compartment, where the drug was loaded into the donor chamber and aliquots were collected from receiver chamber. Liquid chromatography–mass spectrometry (LC–MS) was used to determine the concentration of the drug in the samples. Cumulative amount versus time graphs for passive diffusion and microneedle-mediated diffusion were plotted (Figs. 4–6).

3.3. Transdermal drug flux

Flux values were determined using steady-state portion of the cumulative amount versus time curves [16,17]. Percutaneous penetration of the drug across non-treated pig skin (controlled skin) was low. Passive transdermal drug flux for verapamil hydrochloride and amlodipine besylate were 8.75 μg/cm^2/h and 1.57 μg/cm^2/h respectively. In our study, transdermal flux of verapamil after DermaRoller application was 49.96 μg/cm^2/h (Fig. 7a) while transcutaneous flux of amlodipine besylate following solid microneedle application was 22.39 μg/cm^2/h (Fig. 7b). Drug flux value for amlodipine besylate following passive diffusion was 0.19 μg/cm^2/h when compared to flux value obtained (1.05 μg/cm^2/h) following twelve passes of stainless steel microneedle roller (Fig. 7c).

Fig. 1a. Stainless steel microneedles, density 187 microneedles/cm^2 and 500 μm in length. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 1b. DermaRoller, total-540 microneedles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2b. Displays side view of microneedles.

Fig. 1c. Closer view of cylindrical drum of DermaRoller. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
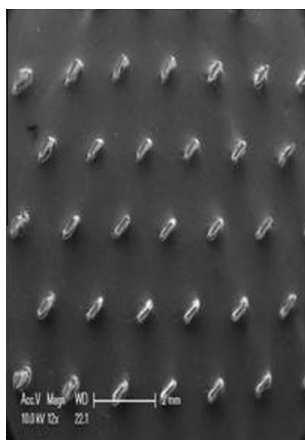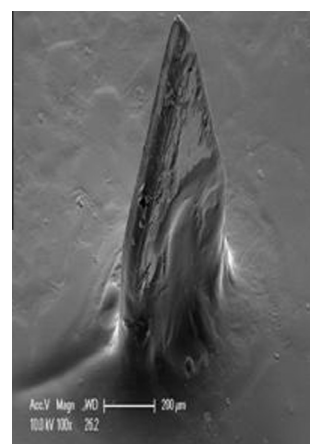

Fig. 2c. Visualized single microneedles.

Fig. 2a. Shows top view of stainless steel microneedles using SEM.
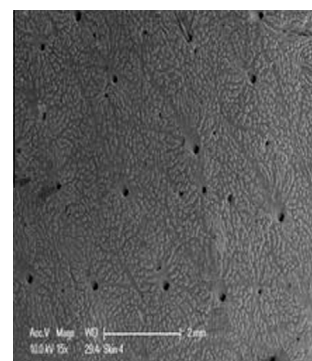
Fig. 3a. Shows microchannels created by using scanning electron microscopy.

4. Discussion

Our results indicate that enhanced permeation of verapamil hydrochloride was statistically significant after the application of microneedle roller. For amlodipine besylate, there was also statistically significant enhancement in transdermal fluxes when solid and microneedle rollers were used. Stainless steel solid microneedles and microneedle rollers were successfully used to breach the epidermis creating microchannels that allowed percutaneous penetration across pig ear skin. Transcutaneous drug delivery is beneficial for patients who face limitations posed by oral or parental drug administration such as first-pass metabolism, gastric irritation and erratic absorption [9,18]. Furthermore, transdermal drug delivery is more advantageous in comparison with bolus delivery method, where full dose of the drug is administered at once. With transdermal patches, there is gradual release of the active ingredient.

When medications are administered transdermally, the drug passes through the stratum corneum, epidermis and dermis. The stratum corneum which is the thin (15–20 μm) and outermost layer of the epidermis poses a major obstacle for transdermal absorption. This layer is made up of corneocytes filled with keratin embedded in a lipid matrix. The lipid matrix consists of ceramides, cholesterol and free fatty acids. Dermis is the innermost layer that contains nerves, blood vessels, lymph vessels, hair follicles, sebaceous glands and sweat glands [19,20]. Meyer et al. showed that

Fig. 3b. Visualize the presence of single microscope using scanning electron microscopy.

Fig. 6. Shows passive and microneedle-assited transdermal permeation of amlodipine besylate after microneedle roller application across pig ear skin (500 μm length, 540 density). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
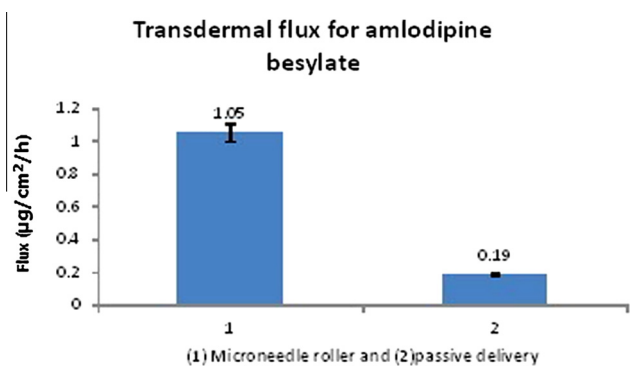

Fig. 4. Shows passive diffusion and microneedle-mediated diffusion of verapamil with microneedle roller across pig skin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 7a. Displays drug flux of verapamil through passive (non-treated skin, controlled) diffusion and microneedle roller treated pig skin (p-value < 0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Displays transdermal penetration of amlodipine across non-treated skin and microneedle-treated skin after solid microneedle was applied (stainless steel solid microneedle, 500 μm length, and density of 187 microneedle/cm²). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 7b. Displays transdermal flux of amlodipine desylate through passive (non-treated skin, controlled) and microneedle-treated skin after application of solid microneedles (stainless steel solid microneedles, 500 μm length, and density of 187 microneedle/cm²) (p-value < 0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

porcine ear skin has structural characteristics similar to human skin. Light microscopy, transmission electron microscopy and cryo scanning electron microscopy methods were used to observe stratum superficiale dermidis of pig ear skin. The results reported by the authors show that pig skin can be used as a model for human integument. In addition, they also reported the storage conditions for pig skin at temperature 4 °C [21]. Our pig skin samples were kept at the same temperature before permeation studies were con-

ducted. Elmahjoubi et al. demonstrated a successful disruption of the stratum corneum by measuring transepidermal water loss using Aquaflux probe. Transepidermal water loss (TEWL) measures the water vapor flux crossing the skin into external environment [22].

Different strategies have been used to increase transdermal permeation of the molecules such as iontophoresis, sonophoresis and chemical penetration enhancers. Iontophoresis uses mild electric

Fig. 7c. Shows transdermal flux of amlodipine besylate across porcine skin using microneedle rollers (*p*-value < 0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

current to deliver charged molecules across the skin. Ita and Banga showed increased transcutaneous absorption of penbutolol sulfate across porcine using iontophoresis. Anodal iontophoresis was used to demonstrate enhancement in transdermal delivery of penbutolol sulfate because the molecule is positively charged [23]. Yamamoto et al. studied in vitro and in vivo permeation of naloxone by anodal iontophoresis. Anodal iontophoresis involves transfer of positively charged molecule across the epidermal barrier by using electrorepulsion. The investigators reported enhancement of in vitro transdermal diffusion of naloxone across pig and rat skin using iontophoresis, which was proportional to current intensity. In addition, in vivo Iontophoretic patches were able to delivery naloxone across dorsal skin of a rat [24]. Even though iontophoresis allows increase in transdermal penetration of small and hydrophilic molecules, it can only deliver molecules smaller than 13 kDa [13]. Wang et al. also discussed about skin irritation caused by iontophoresis at higher electric voltage [25].

Another most commonly used technique used to investigate transdermal permeation studies is the use of chemical penetration enhancers [26,27]. Chemical enhancers are substances that are used to increase the permeability of the skin. Puglia et al. demonstrated effects of polyunsaturated fatty acids (PUFA) and chemical enhancers on permeation of atenolol across human skin [27]. The results of the experiment showed increase in atenolol permeation flux with PUFA and transcutol penetration enhancers. Two polyunsaturated fatty acids tested in their experiments were eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which increased transdermal absorption of atenolol. Lee et al. also investigated the transcutaneous absorption of lidocaine across pig skin and human skin after chemical penetration enhancers were applied. Application of N-methyl pyrrolidone (NMP) and isopropyl myristate (IPM) showed significant increase in transdermal absorption of lidocaine, which improved permeation profile by increasing drug solubility and partitioning into the skin. Although chemical penetration enhancers improve percutaneous absorption of the molecules, many of them cause skin irritations at higher concentrations [28].

Our experiment involved the use of microneedles to investigate the transport of antihypertensive agents across porcine skin. Several variables effect the percutaneous penetration of the molecule across the skin such as microneedle length, microneedle density, and insertion force and insertion time. In addition, microneedle fabrication material and design also impacts transcutaneous delivery of the molecules. In most studies, it has been proven that increased microneedle length and microneedle density enhance permeation of the molecules across epidermis. Li et al. studied transdermal delivery of antibodies using maltose microneedles. It was proven that human IgG delivery was increased when the following

variables were increased-number of microneedle arrays, microneedle length and drug concentration in the donor chamber [29].

In our experiment, stainless steel dermarollers and microneedles were utilized to study the transcutaneous penetration of verapamil hydrochloride and amlodipine besylate. Digital images of stainless steel microneedles and dermarollers are shown in Figs. 1a–1c. Our results demonstrated increased transdermal drug delivery of verapamil hydrochloride and amlodipine besylate in in vitro percutaneous experiments. In addition, scanning electron microscopy was used to visualize microneedles. Li et al. used SEM technique to characterize maltose microneedles and metal dermarollers. The authors also investigated transcutaneous permeation of human Immunoglobulin G across hairless rat skin [29]. Park et al. demonstrated improvement in the transdermal absorption of acetylsalicylic acid across human and porcine cadaver skin using microneedle roller. Microneedle rollers create multiple insertions as the roller rotates across the skin [30]. Zhou et al. showed enhancement in the penetration of insulin on microneedle-roller treated rat skin. The results obtained showed that longer length microneedle rollers led to increased reduction of blood glucose level in comparison with shorter microneedle. The characterization of the microchannels was performed using Evan's blue staining technique [31].

In our investigation, microconduits were visualized using scanning electron microscope. The presence of micropores was showed that microneedles penetrated the stratum corneum. Kumar et al. conducted experiments to test transdermal delivery of calcein and human growth hormone across porcine skin using maltose microneedles. The presence of microchannels was shown by methylene blue staining. In addition, they also determined the uniformity among the micropores through calcein imaging [10]. Banga et al. showed that microchannels created in the skin after the application of the microneedles are large enough to allow the penetration of wide range of molecules as they are in nanometer dimensions. Disruption of the stratum corneum after microneedle application was confirmed by histological sectioning of skin samples [32].

Verapamil hydrochloride and amlodipine besylate have low penetration rates across the skin. Xie et al. conducted experiments that showed transdermal delivery of calcein and bovine serum albumin, which have small molecular structure and hydrophilic nature, respectively. Franz diffusion cells were used to study calcein and BSA diffusion across rat skin using coated microneedles [33]. Park et al. also showed transdermal delivery of calcein, bovine serum albumin and macromolecular proteins using biodegradable microneedles (also known as polymeric microneedles) across human cadaver skin. Successful percutaneous penetration of calcein and human growth hormone across pig skin have been reported where calcein represents a small molecular weight molecule and hGH acts as a larger molecule of 22 kDa [34].

Calcium channel blockers are antihypertensive agents used to manage high blood pressure. To maintain normal blood pressure, L-type voltage gated calcium channels play an important role as they regulate constriction of the vascular smooth muscle and cardiac muscle. Verapamil and amlodipine are L-type calcium channel blockers that belong to distinct chemical classes, phenylalkylamines and dyhydropyridines, respectively [35]. Verapamil has low bioavailability where only 10–20% of the verapamil dose get absorbed from the digestive tract into circulatory system in unchanged form [36]. On the other hand, amlodipine has a bioavailability of 65%, longer half-life and it takes longer for the concentration to reach maximum because of high tissue affinity [37]. Even though amlodipine has benefits if taken orally, it still poses limitations to patients due to gastric irritation, erratic absorption and an initial high plasma concentration.

Some hypertensive patients have poor adherence to existing medications. Most of the tablets are taken in the oral form that passes through liver to get metabolized, known as first pass metabolism effect. In addition, oral medications have higher risk of adverse effects due to variable absorption profile. Patients on chronic conditions are required to take medication on daily basis to manage their condition. Most of them are on therapy that requires taking medication twice or three times a day. Sometimes, patients miss doses of their medications due to amnesia. This can be resolved with transdermal patches. Furthermore, patients' compliance decreases with parental route of drug administration because of needle phobia or needle pain usually caused by conventional hypodermic needles. Li et al. reported that pain induced by microneedles of length 500–1500 µm is relatively low compared to hypodermic needles [29].

Advantages of transdermal delivery include stable plasma concentrations. Administration of the drugs through transdermal patches is free of pain, avoids the risk of transmission of blood borne pathogen and allows patient compliance due to ease of use, especially for elder patients. Microneedle-mediated transdermal drug delivery reduces the chance of infections and skin irritations [2]. Gomaa et al. studied transcutaneous penetration of low molecular weight heparin nadroparin calcium using dissolvable microneedles. Since LMWH are large molecules, hydrophilic and have low bioavailability, they are administered parentally. Parental route of drug administration is associated with pain, and safety issues. According to National patient safety agency, 2716 incidents were reported related to safety concerns. Transdermal drug delivery has proven to be better alternative for administration of LMWH as successful percutaneous delivery was reported by the authors [38].

The results of our study show that the increase in transdermal flux of verapamil hydrochloride across porcine skin following the application of microneedle roller was statistically significant ($p < 0.05$). Amlodipine besylate flux also increased significantly after application of solid microneedles and microneedle rollers ($p < 0.05$). Further in vitro studies will be conducted to test other variables that effect transdermal dug flux such as microneedle design, length, density, and drug concentrations. In addition, different techniques such as iontophoresis, sonophoresis and chemical enhancers can be combined with microporation to study the effect on percutaneous penetration of these antihypertensive agents. Future studies would also involve in vivo studies to investigate the efficiency of transdermal drug delivery systems for verapamil hydrochloride and amlodipine besylate.

5. Conclusion

Microneedle-mediated transdermal drug delivery is beneficial for patients with chronic conditions such as hypertension, diabetes and Alzheimer's disease. Transdermal patches are convenient for patients as it allows for self-administration and it is a pain-free process that produces no biohazardous waste. Due to these advantages, it has the capability to improve patient's adherence to pharmacotherapy. The results of this study show that application of stainless steel microneedles increased transdermal delivery of verapamil hydrochloride and amlodipine besylate across porcine ear skin. These results show that transdermal patches can be developed for these antihypertensive drugs.

Acknowledgements

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