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Influence of salivary washout on drug delivery to the oral cavity using coated microneedles: An *in vitro* evaluation

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ABSTRACT

The objective of this study was to determine whether in buccal tissues, after insertion and removal of coated microneedles, the presence of saliva over the insertion site can lead to loss of the deposited drug, and if saliva can influence *in vitro* permeation of the drug across the tissue. Microneedles were coated with sulforhodamine (SRD), which was used as a model drug, and inserted in to porcine buccal mucosa *in vitro*. Fluorescence microscopy was used to study microneedle coating quality and the diffusion of SRD through the mucosa. Permeation experiments were conducted for simulated dynamic or static salivary flow by adding 100 μL/h or 100, 200 or 300 μL of phosphate buffered saline (PBS) in the donor compartment of the Franz diffusion cells, into which buccal tissue after insertion of SRD-coated microneedles was placed. Microscopy showed that microneedles were uniformly coated with SRD and that SRD was successfully delivered in to the mucosa. Some SRD remained in the tissue even after 24 h, despite presence of PBS on top of the coated microneedle insertion site. It was found that salivary washout can result in loss of drug that has been deposited in oral cavity mucosal tissues using coated microneedles, and presence of fluid over the coated microneedle insertion site can increase flux across the tissue. Thus, it is advisable to include salivary flow during *in vitro* studies related to the use of coated microneedles for drug delivery to the oral cavity in order to not obtain misleading results.

1. Introduction

The oral cavity mucosa has been noted as an interesting site for drug delivery of topically applied formulations. The transmucosal route offers several advantages such as fast onset of action (increased blood supply); absence of drug degradation as seen in the gastrointestinal tract; absence of hepatic first pass metabolism; reduced dose and toxicity; and potential to achieve local or systemic therapeutic effects (Hassan et al., 2010; Patel et al., 2011).

Despite the higher permeability of the oral mucosa in comparison to that of the skin (Squier et al., 1991; Lesch et al., 1989), it's outermost layer, the stratified squamous epithelium, represents a significant challenge in drug delivery because it acts as an important barrier to drug penetration. Microneedles represent a new approach for topical drug delivery for either local or systemic effects. This system consists of micron-scaled needles, designed to penetrate the barrier and enhance drug delivery in a minimally invasive and painless manner (Gill et al., 2008). Coated microneedles have typically been reported in the literature for drug delivery through the skin (Gill and Prausnitz, 2007a; Gill and Prausnitz, 2007b; Ma and Gill, 2014). However, recently, coated microneedles have also been used to successfully deliver drug across the oral cavity mucosal barrier for different purposes such as immunization (Ma et al., 2014; Zhen et al., 2015; Wang et al., 2015) and oral cancer treatment (Ma et al., 2015).

Besides the presence of an effective barrier to drug penetration, the oral cavity is a moist environment with a salivary flux, which constantly washes the oral mucosa, dilutes the drug, and can reduce the contact of a topically applied formulation and its bioavailability, a phenomenon known as “saliva wash out” (Patel et al., 2011; Paderni et al., 2012; Chinna Reddy et al., 2011). Thus, keeping the formulation on its application site for longer duration, and minimizing its loss due to salivary flow, is a great challenge.

In spite of the presence of saliva in the oral cavity, the efficiency of coated microneedles for drug delivery in to oral cavity tissues has been reported to be comparable to that in the skin, which is a dry surface. Ma et al. have reported delivery efficiencies of 63.9% ± 6.9% and

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91.2% ± 1.6% into the lip and tongue of a rabbit, respectively (Ma et al., 2014). However, McNeilly et al. have reported a much lower delivery efficiency of 31.7 ± 3.7% into the mouse buccal tissue (McNeilly et al., 2014). While these studies have quantified the amount of drug delivered into the mucosa using coated microneedles, it remains unclear whether the drug that is deposited into the tissues can be backwashed due to saliva that bathes the insertion site, and whether presence of saliva can affect the diffusion of the deposited drug deeper into the tissue.

Thus, we were motivated to determine the effect of saliva on the drug that is deposited into the oral cavity tissues *via* coated microneedles. *In vivo* studies to determine this effect can be complicated and tough to interpret. This is because drug lost, if any, from salivary flow will be ingested by the animal, and thus cannot be quantified directly. Thus, we simulated the salivary flux condition *in vitro* using a Franz diffusion setup. The Franz diffusion is a classical experiment that is widely used to evaluate drug release and permeation across different barriers such as the skin or other different mucosal tissues including the oral buccal tissues. Usually, both pig buccal mucosa and skin are used to simulate the respective human tissues due to their high similarities in terms of permeability, structure and composition (Lesch et al., 1989). Typically, *in vitro* permeability studies using a Franz diffusion setup involve addition of a buffer or a formulation containing the drug over the tissue, in conjunction with either a permeability enhancing agent added to the formulation or after pretreatment of the tissue to increase its permeability. However, coated microneedles are unique because they directly deposit the drug into the tissues. Thus, further assessment of diffusion of this deposited drug across the tissue should not involve addition of fluid in the donor chamber in the case of skin. On the contrary, for oral cavity tissues, saliva should be simulated in the donor chamber. Nonetheless, to our knowledge, there are no studies that have examined *in vitro* permeability across either the oral cavity mucosa or the skin for drug that has been deposited in the tissues using coated microneedles.

Therefore, the objective of the present study was to test the hypothesis that it is possible to perform an *in vitro* permeation study in a Franz-type vertical diffusion cell with porcine buccal mucosa into which drug has been delivered using coated microneedles, and, to simulate salivary flow *in vitro* to evaluate the influence that saliva has on drug loss and drug permeation across the buccal tissue.

2. Materials and methods

2.1. Microneedles

According to a previously described method (Ma and Gill, 2014; Ma et al., 2014), a wet etch process was used to fabricate 2D microneedle patches comprising of 57 microneedles (700-μm long and 200-μm wide) from a 50 μm-thick stainless steel sheet (SS304). As described previously (Gill and Prausnitz, 2007a), each microneedle of the 2D patch was bent "out of plane" manually under a microscope.

Microneedles were coated using a micro-precision dip coating process (Gill and Prausnitz, 2007a; Ma et al., 2015; Ma et al., 2014). Briefly, an automated x-y linear computer-controlled device on which microneedle arrays were positioned, was used to dip microneedles into the coating solution. The coating solution was composed of 1% (w/v) of carboxymethylcellulose sodium salt (low viscosity, USP grade, CarboMer, San Diego, CA, USA), 0.5% (w/v) Lutrol F-68 NF (BASF, Mt. Olive, NJ, USA) and 0.25% (w/v) sulforhodamine (SRD) (Molecular Probes, Eugene, OR, USA) (Ma et al., 2014).

2.2. Preparation of porcine buccal mucosa

Porcine buccal mucosa was obtained from Innovative Research (Novi, MI, USA). The excess of underlying tissue was manually removed with scalpels and scissors, until the samples had about 1.5 mm thickness, which was measured with a caliper. After preparation, the samples were kept frozen (−80 °C) for no longer than 3 weeks.

Before all experiments, to ensure tissue integrity, electrical impedance across mucosa was measured using a LCR Meter (LCR200, EXTECH Instruments, Nashua, NH, USA). First the mucosal tissue was cut to size and mounted on the Franz diffusion cell with phosphate buffered saline (PBS) in the donor and in the acceptor chambers. Next the two electrodes were placed in the donor and acceptor chambers, respectively. Mucosa was considered reliable with resistivity higher than 2 kohm·cm^2. This resistivity value was obtained based on a previous study by de Vries et al. (de Vries et al., 1991), which we verified through pilot studies. In our pilot studies, porcine buccal mucosa were prepared and punctured with hypodermic needles. Impedance values of these tissues before and after puncture were measured. Resistivity values of non-punctured tissues were >2 kohm·cm^2, while the punctured tissues had lower values.

2.3. Characterization of coated microneedles and delivery into porcine buccal mucosa in vitro

Fluorescence stereomicroscope (Olympus SZX16 fitted with DP73 CCD camera, Olympus America Inc. fitted) was used to visually inspect uniformity of coatings on the microneedle surface and to inspect microneedles before and after insertion into the porcine buccal mucosa. For insertion, microneedles coated with SRD were manually pressed into the porcine buccal mucosa and held in place for 5 min. After a 5-min period, microneedles were removed and inspected under a fluorescent stereomicroscope. The surface of the porcine buccal mucosa after insertion was also visualized under the microscope. The porcine buccal mucosa was next placed in OCT compound (Tissue-Tech, 4583, Sakura Finetek, Torrance, CA, USA), and frozen (−80 °C). The samples were sliced into 10-μm thick sections using a cryostat (CM 1950, Lec, Buffalo grove, IL, USA). Fluorescence microscopy images were obtained for these sections using an inverted fluorescent microscope (Nikon Ti eclipse fluorescent microscope) fitted with a CCD camera (Andor DR-328G-c10-SIL, Andor Technology, South Windsor, CT, USA).

2.4. Determination of delivery efficiency of coated microneedles

Transmucosal delivery efficiency (DE) of coated microneedles was determined according to a previously described methodology (Gill and Prausnitz, 2007a; Ma et al., 2014; Ma et al., 2015). The amount of drug delivered into the mucosa was calculated by subtracting SRD that remained on microneedles after mucosal insertion (C_2 – in μg/mL) and SRD that remained on top of the mucosal surface (C_3 – in μg/mL) from the total amount of SRD that was coated on microneedles (C_1– in μg/mL), and DE was found according to the equation:

$$DE = \frac{C_1 - (C_2 + C_3)}{C_1} \times 100$$

For all these measurements freshly prepared SRD-coated microneedles were used. Briefly, an unused patch of SRD-coated microneedles was inserted in 1 mL of deionized water for 15 min to quantify the amount of SRD on the coating (C_1). Next, another set of coated microneedles were applied on top of the mucosal surface for 5 min, and SRD that remained on microneedles was obtained by placing the used patch in 1 mL of deionized water to determine C_2. The amount of drug left on the tissue surface was gently removed with a moistened swab followed by its immersion in 500 μL of deionized water to quantify C_3. Samples were analyzed using a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Santa Clara, CA, USA) at the excitation and emission wavelengths of 565 and 586 nm, respectively, together with a standard curve of SRD.