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Formulation and characterisation of dissolving microneedles for the transdermal delivery of therapeutic peptides

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ABSTRACT

The highly effective barrier properties of the stratum corneum (SC) limit the application of transdermal delivery to relatively small, lipophilic molecules. Microneedles (MNs) however, offer a route to effectively deliver a wide range of pharmaceuticals through the skin, bypassing the SC in a non-invasive and pain-free manner. This study presents a dissolving MN system composed of polyvinylpyrrolidone (PVP) and trehalose to encapsulate active pharmaceutical peptides within the MN matrix. Rapid systemic delivery is then achieved once the needles have penetrated the SC and dissolved in the interstitial fluid of the skin. A variety of characterisation techniques were carried out to determine the optimum formulation. A model peptide, polymyxin B, was then incorporated into the MN system and delivered through porcine skin. In addition, the activity of the model drug was monitored during all stages of the formulation process.

1. Introduction

Compared with conventional therapeutics, peptides have many advantages including high activity, high specificity and typically are effective at low concentrations (Renukuntla et al., 2013; Chandrudu et al., 2013; Castro et al., 2015). In addition, peptides degrade into amino acids which are far less biologically harmful than the toxic metabolites which can accumulate in the body, often as a result of the metabolism of small molecule drugs (Tsomaia, 2015). To date, peptides have been employed therapeutically in a wide range of areas including neurology, endocrinology and haematology (Edwards et al., 1999a). The increase in targeting metabolic disorders with peptides has resulted in the development of longer and more complex molecules. However, interest continues in the development of shorter peptides with less than 10 residues in the area of peptide based vaccines (Lax, 2012), which highlights the scope of these therapeutic molecules.

Given their poor oral bioavailability, peptides were often overlooked as potential therapeutic agents, however, pharmaceutical companies have since come to accept the potential of orally unavailable drugs (Vlieghe et al., 2010). Research continues into novel analogues and delivery methods with inhaled, buccal, intranasal, rectal and sub-lingual preparations of the drugs being explored (Edwards et al., 1999a, 1999b). Recent advances in the areas of molecular biology, immunology and enzymology, in addition to progress in synthetic biotechnology, have increased the potential for peptide use in pharmaceutical product development (Anon, 1999). Similarly, the development of pharmaceuticals using recombinant technology and genetic engineering, in addition to creating a surge of interest in therapeutic peptides, also initiated a major focus on developing alternative drug delivery systems to injections (Lax, 2012).

Transdermal drug delivery devices are an attractive alternative to invasive injection-based delivery methods. The SC provides the main barrier to skin permeation and as such, limits the therapeutic effectiveness of any topically applied compounds (Benson and Watkinson, 2011; Wermeling et al., 2008). The rate of percutaneous drug transport across the skin is directly limited by the SC, which is much more effective as a barrier to drug delivery than the epithelial barriers of the GI, buccal, nasal, vaginal or rectal delivery paths (Ratna, 2004). Any permeation is limited to lipophilic or hydrophobic compounds due to the SC's high lipid content and, as the SC is effectively composed of dead tissue, the mode of transport is via passive diffusion. In contrast, the viable epidermis is hydrophilic and as such, acts as a rate limiting barrier in the case of particularly lipophilic compounds (Anon, 2012). However, in general, once the drug molecules have passed through the SC layer, passage into the other dermal layers and subsequent

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systemic delivery occurs quite rapidly and with ease (Ratna, 2004; Davidson et al., 2008).

MN technologies have emerged as a practical and less invasive methods of overcoming the barrier properties of the SC and delivering drugs effectively across the skin, including high-molecular weight and/or hydrophilic drugs (Prausnitz and Langer, 2008; Donnelly et al., 2010). Akin to a hybrid system of both hypodermic needle and transdermal patch technologies, MN's consist of an array of micron sized needles (Bal et al., 2010; Martin et al., 2012), which when applied to the surface of the skin, breaches the SC and epidermis, typically without reaching the pain receptor nerves in the deeper tissues of the dermis (Anon, 2010; Kaushik et al., 2001). Diffusion of the drug then occurs through the epidermis, via the microconduits created by the needles, to the dermis from where the drug is then absorbed by capillaries into the bloodstream. The microconduits created by the arrays allow higher molecular weight compounds to penetrate the skin barrier that would normally be unable to passively permeate through (Bal et al., 2010).

Dissolving MNs are designed to completely dissolve in the interstitial fluid of the skin during the application process (Kim et al., 2012) and are typically fabricated from biocompatible and biodegradable materials which house the drug payload within the matrix of the MNs themselves (van der Maaden et al., 2012). Depending on the constituents used, dissolving MN's can fully degrade within 5 min in the case of water soluble materials or may take several days for some biodegradable polymers (Kim et al., 2012). Studies have utilised a wide range of materials for dissolving MN systems including trehalose (McGrath et al., 2014) maltose (Li et al., 2009), carboxymethylcellulose (CMC) (Lee et al., 2008), PVP (Sun et al., 2013; Ke et al., 2012), poly(lactic-co-glycolic) acid (PLGA) (Park et al., 2005), hyaluronic acid (Liu et al., 2012), and fibroin (You et al., 2011).

Peptides, proteins and antigens are all sensitive to temperature and require benign formulation conditions to avoid damaging their pharmaceutical activity (Vecchione et al., 2014). As such, encapsulation and solidification of MNs incorporating these drugs must be carried out at lower temperatures and in many cases, avoiding harsh environments such as high or low pH, high pressure and organic solvents (Anon, 1999). This study presents a dissolving MN system composed of biocompatible polymers and stabilising sugars to encapsulate active pharmaceutical peptides within the MN matrix. Rapid and systemic delivery is then achieved once the needles have penetrated the SC and dissolved in the interstitial fluid of the skin.

2. Materials and methods

2.1. Materials

PVP with average molecular weight of 40 kDa was supplied by Sigma Aldrich. D(+)-trehalose dihydrate was obtained from Fisher Scientific Ireland. Trypan Blue was obtained from Sigma Aldrich Ireland. Glycerol – ACS grade was obtained from Reagecon. Polyethylene glycol (PEG) 400 was supplied by Merck-Schuchardt. Poly (lactic-co-glycolic) acid (PLGA) was obtained from Purac Biomaterials and Sylgard ® 184 polydimethylsiloxane (PDMS) elastomer kit was supplied by Fisher Scientific Ireland. Polymyxin b sulphate was supplied by Sigma Aldrich. Fresh, untreated pig ears were kindly supplied by Dawn Meats, Grannagh, Waterford, Ireland and stored at −20 °C prior to use. *Salmonella typhimurium* (LT2) was obtained from Waterford Institute of Technology (WIT) culture collection. Phosphate buffered saline (PBS) solution was obtained from Fisher Scientific. Brain heart infusion (BHI) agar was supplied by Oxoid Limited.

2.2. Fabrication and characterisation of PVP/trehalose MN's

The MN master moulds were designed and fabricated using the Xtreme Laser Facility (XLF) in Cardiff University by Professor David Barrow. To fabricate the moulds, silicon sheets were laser ablated to create pre-programmed inverse patterns of the arrays. The MN design used consisted of an array of 18 × 18 needles each with a shaft length of 650 μm, 200 μm base width and 600 μm spacing between needles.

PLGA MN moulds were fabricated from the silicon master moulds by placing PLGA granules over the moulds which were then heated to 150 °C in a vacuum oven at low vacuum (<5 mbar) for 1 h. Following this, a batch of PDMS was prepared using a Sylgard ® 184 elastomer kit. The PDMS was then poured over the PLGA moulds in a petri dish and then placed in an oven to cure at 100 °C for 45 min. Once the PDMS had set, the PLGA moulds were carefully peeled away leaving PDMS inverse moulds which would be used to cast the polymer/sugar MN arrays.

For each formulation, the polymer and sugar components were dissolved in 10 ml of deionised (DI) water and the plasticiser was then added. Once fully dissolved, the formulation was pipetted over the PDMS MN moulds ensuring total coverage of each mould. The formulations were then held under vacuum to fill the mould cavities and cast the MN arrays.

The sugar/polymer formulation was then carefully applied directly onto the micromould surface and the micromoulds were placed in a vacuum oven set at 22 °C with the vacuum pressure set to 5 mbar for 15 min. After this, the vacuum was released and the moulds were removed. The formulation was gently agitated on the mould surface to remix the formulation and remove any bubbles from the formulation droplet. The moulds were returned to the oven and the process was repeated until the formulations ceased bubbling under vacuum, indicating that the mould cavities had been fully filled by the formulation. The vacuum was then released and the micromoulds were placed in an incubator at 22 °C and left for 24 or 48 h to dry, depending on the formulation. Once the formulations had dried sufficiently, each MN array was carefully peeled off the PDMS mould and the entire batch was then stored in a desiccator prior to subsequent analysis. The MN arrays produced were approx. 12 mm^2 with an average weight of 105 mg.

2.3. Scanning electron microscopy (SEM)

The instrument used was a Hitachi S-2460N SEM. For sample analysis, the emission current was set to 80 μA and the accelerating voltage was set to 22 kV. Prior to SEM analysis, each sample was affixed to a metal stub using carbon adhesive tabs before being gold coated using a K550 Emitech Sputter coater with deposition current set to 35 mA and a coating time of 3.5 min.

2.4. Thermogravimentric analysis (TGA)

The instrument used was a TA Instruments Q50 TGA. A standard heating programme was selected incorporating a heating rate of 10 °C/min from ambient temperature up to 500 °C with the balance purge flow set to 50 ml/ min and the sample purge flow also set at 50 ml/min. All samples were run in triplicate and the thermograms were analysed using TA Instruments Universal Analysis 2000 software.

2.5. Differential scanning calorimetry (DSC)

The instrument used was a TA Instruments Q2000 DSC. All formulations were tested in triplicate with sample weights ranging from 5 to 10 mg. All samples tested were placed into Tzero aluminium pans which were hermetically sealed. A pin hole was