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Research paper

Evaluation of geometrical effects of microneedles on skin penetration by CT scan and finite element analysis

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ABSTRACT

Computerized tomography scan (CT scan) imaging and finite element analysis were employed to investigate how the geometric composition of microneedles affects their mechanical strength and penetration characteristics. Simulations of microneedle arrays, comprising triangular, square and hexagonal microneedle base, revealed a linear dependence of the mechanical strength to the number of vertices in the polygon base. A laser-enabled, micromoulding technique was then used to fabricate 3×3 microneedle arrays, each individual microneedle having triangular, square or hexagonal base geometries. Their penetration characteristics into *ex-vivo* porcine skin, were investigated for the first time by CT scan imaging. This revealed greater penetration depths for the triangular and square-based microneedles, demonstrating CT scan as a powerful and reliable technique for studying microneedle skin penetration.

1. Introduction

Microneedles are minimally invasive devices that facilitate transdermal drug delivery by creating micron size pores through the skin [1–4]. Due to its viscoelastic nature [5], skin is easily deformed when microneedles are applied to its surface, and therefore, the microneedle design and constructional material will determine the extent of skin penetration by the microneedles, and in the resulting effect on drug permeability [2]. The most critical factors for microneedle skin penetration were attributed to needle length and density [6,7], tip and base diameter [8–10] and microneedle material [11–13]. In addition, the use of an injection applicator was found to greatly enhance penetration with a critical role on force and velocity of microneedle injection [14–16]. Studies have shown that the penetration depth is linearly correlated with microneedle length and inversely correlated with microneedle density. The bed-of-nails effect begins to have a negative effect on penetration, at interspacing values smaller than 150 μm [14,15,17]. Furthermore, microneedles made from material of high Young's modulus, showed enhanced mechanical properties and penetration characteristics [12,13].

In order to accurately evaluate skin permeability by microneedles of different geometries, it is important to use highly accurate and reproducible techniques. Traditionally, histological sectioning has been employed to determine the depth of penetration. This involves freezing and sectioning skin that has been treated with microneedles [18,19]. In addition to being a cumbersome technique, histological processing carries the risks of errors occurring. This can be during the selection of the sampling area, and also, of possible alterations in the structure of the skin after the microneedles are removed, as expected for hyperelastic materials such as the skin [20,21]. To limit the problems associated with histological processing, whole tissue techniques such as confocal microscopy [12,22,23] and optical coherence tomography (OCT) [15,24,25], are often implemented. Both techniques offer the advantage of *in vivo* application, with OCT having the added benefit of achieving higher penetration depths of approximately 2.0 mm, compared with 0.25 mm of confocal microscopy [15].

In this study we introduce CT scanning as an alternative, non-destructive powerful technique for studying, in detail, microneedle skin penetration through 3D visualization. CT scanning produces 3D volumetric data that are combined from a series of X-ray

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images taken at different rotation angles. This renders it a popular imaging technique for use in medicine, archaeology, geology and material sciences [26]. In this work, we demonstrate the application of CT scanning for drug delivery research, by studying the skin penetration characteristics of triangular, square and hexagonal based microneedles. Microneedle arrays, of 3 × 3 individual microneedles, were prepared using a highly controlled and versatile, laser-enabled, micromoulding technique and structural mechanic simulations were employed to assess their mechanical properties by determining the average von Mises stresses and critical buckling loads.

2. Methods

2.1. Simulations

Simulations were performed using the Structural Mechanics Module of COMSOL Multiphysics version 4.2b (www.comsol.com). Simulations were performed on single microneedles with the following dimensions: footprint diameter (200 μm), height (800 μm), tip diameter (10 μm). Regular polygons (triangle, square, hexagon) were used as 2D base shapes using a polygon vertex calculator (http://www.mathopenref.com/coordpolycalc.html) to obtain the Cartesian graphic coordinates of each polygonal vertex. The R_e (radius of circle encompassing base) remained at 100 μm and this data was manually entered into COMSOL Multiphysics software using the polygonal tool to create a 2 dimensional base shape for each of the microneedles. These shapes were then linearly extruded 800 μm in the Z axis with scale factors in the X and Y directions of 0.05, yielding a tip diameter of 10 μm and a fixed tip angle. The triangular, square and hexagonal microneedles, as modelled in the single needle, were each formed into 3 × 3 microneedle arrays with 600 μm needle-to-needle spacing, using the Array function in COMSOL Multiphysics. A square backing plate (3 mm × 3 mm × 0.5 mm) was joined to the bases of all 9 microneedles, for each of the three arrays. A linear elastic model using the Young's modulus (3.5 GPa) and density (1.24 g/cm^3) values of PLGA (provided by PURAC Biomaterials) was adopted for the microneedles. The estimated value for Poisson's ratio was 0.3. The skin was simulated as two cylindrical structures, the top cylinder emulating the stratum corneum and viable epidermis using 1 MPa as the Young's modulus [27] value and having dimensions of 600 μm in diameter, 100 μm in height and the bottom cylinder emulating the dermis using 0.066 MPa [28] as the Young's modulus value and 600 μm in diameter, 1000 μm in height. Both epidermis and dermis were treated as nearly incompressible materials and the Poisson's ratio was set at 0.495. The Structural Mechanics module of COMSOL Multiphysics was used to perform stationary and linear buckling analyses with a 5 N applied force details of which, are described in previous work [12].

2.2. Microneedle array fabrication

The 3 × 3 inverse microneedle moulds were prepared on a sheet of polydimethylsiloxane (PDMS) by laser ablation, using an Exitech Model 2000EF, Microablator. This incorporates a Thales femtosecond laser source operating at 795 nm, with a 5 kHz repetition, and a pulse width of 125 fs. This light was focused at the workpiece, producing a spot of approximately 1.5 μm. All ablation patterns were designed using the software Alpha CAM V5. 3 × 3 arrays of regular polygons (triangles, square, hexagons) were designed with outer circle diameter of 200 μm, centre-to-centre spacing of 600 μm and distance between cuts of 25 μm. The laser power was 0.5 W at the work-piece. The PDMS moulds were thoroughly cleaned by washing with ethanol and sonicating in water for 1 h and subsequently, used to prepare microneedles made of PLGA (DL-lactide/glycolide copolymer, PURAC Biomaterials) using a high temperature, vacuum deposition method. A typical procedure involves depositing solid PLGA on top of the micromould and then placing this in a vacuum oven at 160 °C for 60 min to melt the PLGA granules. The vacuum was then applied for a further 30 min before being slowly released so as to force the molten polymer into the micromould. Subsequently, the moulds were removed from the oven and allowed to cool for 10 min at room temperature to solidify the PLGA. These were then cooled for a further 10 min at −20 °C. The solid PLGA microneedles were then carefully removed from the mould. All PLGA microneedles were sputter-coated with 100 nm of gold using a Baltec Sputter coater, prior to imaging with the CT scanner.

2.3. CT scan imaging

Three-dimensional images of both stand-alone microneedles and microneedle arrays inserted into porcine skin, were obtained using a Bruker microCT SkyScan1174. This comprised an RTW 50/800 X-ray source, with an adjustable anode voltage between 20 and 50 kV, and a current of up to 800 μA. Specimens were mounted on polystyrene discs, 12 mm in diameter, using double-sided adhesive carbon discs and fitted on the CT scanner's specimen table. In all instances, flat field references were acquired prior to scanning, and random movement selected to compensate for ring artefacts. The source voltage and current were set to 50 kV and 800 μA, respectively and scans were collected at 360 deg. Exposure time and rotation steps were set to 60 ms and 0.050 deg, for stand-alone microneedles, and 800 ms and 0.100 deg for microneedles in skin. Standard mode reconstruction was performed using NRecon, version: 1.6.9.4 and further image processing using CTVox and Bruker's DataViewer software.

3. Results

3.1. Simulations

The mechanical properties (von Mises stress and critical buckling load) of the different geometry microneedles, were computationally assessed, using the Structural Mechanics module of COMSOL Multiphysics. The von Mises stress refers to the combined principal stresses that act on an object when it is subjected to a system of loads, such as that applied on a microneedle during skin penetration. The critical buckling load refers to the maximum load that a structure can withstand before failing due to buckling. Both these terms can be used to describe and compare mechanical properties of structures of different geometries.

Stationary and linear buckling analyses were performed on 3 × 3 arrays of microneedles, with the following base geometries: triangle, square and hexagon. The results showed an inversely proportional relationship between the number of vertices in the polygon, and the von Mises stress values (Fig. 1). This suggests that as the number of vertices in the polygon structure increases, the microneedles can withstand higher compressive loads; i.e. hexagonal based microneedles are less likely to fail from fracture when compared to triangular based microneedles. On the other hand, the predicted critical load factor, k, associated with the linear buckling analysis, showed a proportional relationship to the number of vertices in the polygon. The critical load factor, k, refers to the ratio of buckling load, and is an indicator of the factor of safety against buckling. Thus, the higher the value of k, the lower the risk of buckling. The k values are at the highest for the hexagonal base microneedles and at the lowest for triangular base microneedles (Fig. 1).