Effects of Microneedle Shapes on the Efficiency of Microneedle-mediated Vaccine Delivery: A Finite Element Analysis

ผลของรูปทรงของเข็มขนาดไมโครเมตรต่อประสิทธิภาพของการนำส่งวัคซีน: การวิเคราะห์เชิงไฟไนต์เอลิเมนต์

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ABSTRACT

This study aimed to investigate the influence of microneedle shapes on the efficiency of the microneedle-mediated vaccine delivery using a three-dimensional finite element model. The model describes the antigen release via diffusion and receptor-antigen binding kinetics in the skin. We found that the shapes of the microneedles can affect the APC activations both in the epidermis and the dermis. The results revealed that the rectangular with a sharp tip microneedle provides the highest efficiency of the vaccine delivery, followed by the cylindrical with a sharp tip, the square conical, the arrowhead, the circular conical, and the triangular conical microneedles. Due to the fact that the rectangular with a sharp tip microneedle has the largest surface area, it, therefore, can hold the most substantial amount of the antigen and activate the highest number of APCs.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาอิทธิพลของรูปทรงเข็มขนาดไมโครเมตรต่อประสิทธิภาพการนำส่งวัคซีนด้วยแบบจำลองไฟไนต์เอลิเมนต์สามมิติ โดยแบบจำลองอธิบายถึงการปลดปล่อยและการแพร่ของแอนติเจน รวมถึงกลไกการจับตัวกันของแอนติเจนและตัวรับของเซลล์ในผิวหนัง เราพบว่ารูปทรงของเข็มขนาดไมโครเมตรส่งผลต่อการกระตุ้นเซลล์นำเสนอแอนติเจนในผิวหนังทั้งในชั้น epidermis และชั้น dermis ผลการศึกษาแสดงให้เห็นว่าเข็มรูปทรงก้านสี่เหลี่ยมปลายแหลมมีประสิทธิภาพสูงที่สุด ตามด้วยก้านทรงกระบอกปลายแหลม กรวยฐานสี่เหลี่ยม ลูกศร กรวยฐานกลม และกรวยฐานสามเหลี่ยม ทั้งนี้เนื่องจากเข็มขนาดไมโครเมตรรูปทรงก้านสี่เหลี่ยมนั้นมีพื้นที่ผิวมากที่สุด จึงส่งผลให้มีปริมาณของแอนติเจนเคลือบอยู่ที่ผิวมากที่สุด และปล่อยแอนติเจนเข้าสู่ผิวหนังในปริมาณมากที่สุด เมื่อการกระตุ้นการตอบสนองของภูมิคุ้มกันขึ้นอยู่กับปริมาณของแอนติเจนที่ถูกนำเสนอต่อเซลล์ภูมิคุ้มกัน จึงทำให้การตอบสนองต่อภูมิคุ้มกันจากการใช้เข็มรูปทรงนี้มีค่าสูงที่สุด

Keywords: Coated microneedle, Transdermal vaccine delivery, Inducing immune response
คำสำคัญ: เข็มขนาดไมโครเมตรประเภทเคลือบผิว การนำส่งวัคซีนผ่านผิวหนัง การกระตุ้นการตอบสนองทางภูมิคุ้มกัน

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Introduction

Owning to the fact that the conventional transdermal vaccine delivery is limited by the outermost layer of the skin, the stratum corneum (SC) (Prausnitz et al., 2009), microneedles (MNs) have been increasingly utilized to enhance transdermal delivery of small and large molecules. The microneedles can create very small pores in the skin and deliver the vaccine into the skin directly. They can, therefore, increase skin permeability and reduce pain for patients. Furthermore, the array of MNs can provide a sustained release of medication into patients (Li et al., 2019). In addition, recent studies reported that the epidermis and dermis layers of the skin are rich in dendritic cells, which are robust antigen presenting cells (APCs). The APCs are known as Langerhans cells and dermal dendritic cells in the epidermis and the dermis, respectively. These cells are important in immunostimulatory and migration activities. They consequently play a major role in adaptive immune response (Lambert et al., 2008). The transdermal delivery of vaccines is, therefore, one of the most effective means of controlling infectious diseases.

Recently, microneedles in a variety of geometries, including circular cone, square cone, triangular cone, cylinder with a sharp tip, rectangular with a sharp tip, and arrowhead shapes, have been designed and fabricated in laboratory (Johnson et al., 2016; Kavaldzhiev et al., 2017; Samant et al., 2018). These microneedles have been clinically used for hormonal therapy, insulin delivery in diabetes patients, and delivery of contraceptives (Li et al., 2019). In addition to a laboratory-design approach, a modeling and computational approach has also been employed to optimize the geometry of microneedles. Devidson et al. studied the geometry effects of microneedles on the effective skin thickness and the drug permeability (Davidson et al., 2008). They found that the depth of penetration from microneedle array is the most important factor, while the microneedle diameter and coating depth are less significant. Recently, Römgens et al. predicted the optimum geometry of microneedles and their array for dermal vaccination using a finite-element modeling approach (Römgens et al., 2016). They found that the optimum distance related to the number of activated APCs. The optimum distance depended on the antigen dose. The microneedle length affected the number of activated APCs in the epidermis and the dermis. However, to the best of our knowledge, there is no study on the effects of shapes of microneedles on the efficiency of vaccine release and their potential on the induced immune response.

In this work, a Finite Element Analysis of microneedle-mediated vaccine delivery was carried out. Our finite element model takes into account the diffusion of antigens, the skin kinetics of antigen-receptor binding, the internalization of antigens into cells, and the uptake of antigens by blood vessels. Six microneedle shapes, namely, a circular cone, a square cone, a triangular cone, a cylinder with a sharp tip, a rectangle with a sharp tip, and an arrowhead, were simulated with the aim for finding the most effective microneedle shape in inducing an immune response.

Objectives of the study

The aim of this study was to investigate the roles of microneedle shapes on the efficiency of vaccine delivery using a finite element modeling approach.

Methodology

Model geometry

In this study, microneedles in a microneedle patch are arranged in a square array with a center-to-center distance S of 1 mm. Due to the symmetry of the array, modeling only a single microneedle (Figure 1) is sufficient for investigating the efficiency of the microneedle. Here, six microneedle shapes, namely, a circular cone, a square cone, a triangular cone, a rectangle with a sharp tip, a cylinder with a sharp tip, and an arrowhead, were investigated. The microneedle geometry is described by the microneedle length l_{mn} and the base radius r_b. The default values of l_{mn} and r_b for each microneedle shape are summarized in Table 1.

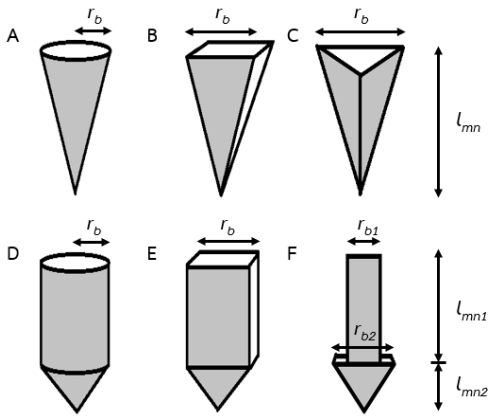

Figure 1 Microneedle shapes. (A) a circular cone, (B) a square cone, (C) a triangular cone, (D) a cylinder with a sharp tip, (E) a rectangle with a sharp tip, and (F) an arrowhead

Table 1 The model parameters used to describe each microneedle geometry

Microneedle shape	Microneedle length (mm)	Base radius/width (mm)
Circular cone	0.52	0.13
Square cone	0.52	0.26
Triangular cone	0.52	0.26
Rectangular with a sharp tip	0.4/0.13	0.26
Cylinder with a sharp tip	0.4/0.13	0.13
Arrowhead	0.4/0.17	0.13/0.17

The microneedle models (Figure 2) were implemented using COMSOL Multiphysics version 5.3. The microneedle is coated with an antigen with a coating thickness t_c of 0.02 mm around the microneedle. The microneedle sticks through the skin with the thickness t_e of 0.2 mm and t_d of 1.8 mm for the epidermis and the dermis, respectively. To minimize the numerical error that might occur around the interface boundaries, the fine mesh element was used around these regions. However, the coarse mesh element toward the edges was used to reduce the time required for simulating the models.

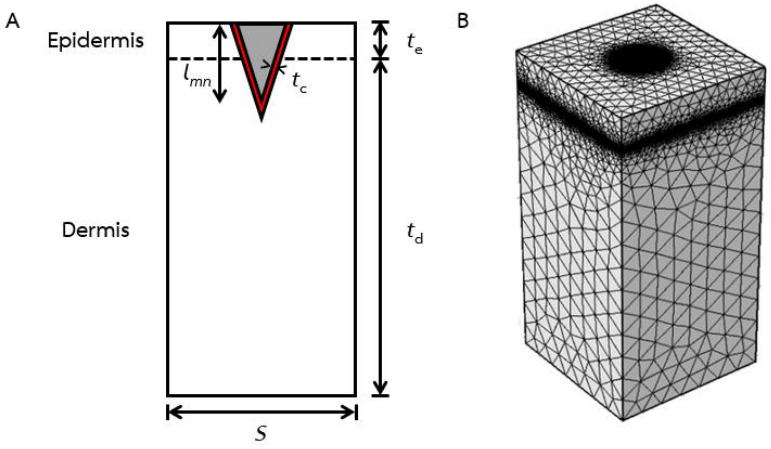

Figure 2 Schematic representation of the microneedle and skin geometry. (A) The microneedle has a length l_{mn} and a coating thickness t_c. The skin is divided into the epidermis and the dermis with a thickness t_e and t_d, respectively. (B) The finite mesh element

Mathematical model of vaccine transport and skin kinetics

We adapted the mathematical model describing the vaccine transport and skin kinetics proposed earlier by Römgens and co-workers (Römgens et al., 2016). After the microneedle penetrates the skin, the coated antigen starts to release to the skin via diffusion. Antigen presenting cells (APCs), Langerhans cell in the epidermis and Dermal dendritic cell in the dermis, bind and internalize these antigens into the cells via receptor-mediated endocytosis. In addition, the antigens can be taken up into blood capillaries located in the dermis layer. A set of partial differential equations (1) - (6) describes rates of change of concentration as a function of time t and coordinate x, y, and z. These equations describe the process of antigen release, diffusion, antigen-receptor binding, and internalization by the APCs. The default parameter values used to describe the kinetics are summarized in Table 2.

$$\frac{\partial C_{MN}}{\partial t} = \nabla(D_{MN}\nabla C_{MN}), \tag{1}$$

$$\frac{\partial C_{\mathrm{ECM},e}}{\partial t} = \nabla(D_e\nabla C_{\mathrm{ECM},e}) - k_a C_{\mathrm{ECM},e}(R_{\mathrm{tot},e} - C_{\mathrm{rec}} - C_{\mathrm{cells}}) + k_d C_{\mathrm{rec}}, \tag{2}$$

$$\frac{\partial C_{\mathrm{ECM},d}}{\partial t} = \nabla(D_d\nabla C_{\mathrm{ECM},d}) - k_a C_{\mathrm{ECM},d}(R_{\mathrm{tot},d} - C_{\mathrm{rec}} - C_{\mathrm{cells}}) + k_d C_{\mathrm{rec}} - k_c C_{\mathrm{ECM},d}, \tag{3}$$

$$\frac{\partial C_{\mathrm{rec}}}{\partial t} = k_a C_{\mathrm{ECM},i}(R_{\mathrm{tot},i} - C_{\mathrm{rec}} - C_{\mathrm{cells}}) - (k_d + k_{int})C_{\mathrm{rec}}, \tag{4}$$

$$\frac{\partial C_{\text{circ}}}{\partial t} = k_c C_{\text{ECM},e}, \quad (5)$$

$$\frac{\partial C_{\text{cells}}}{\partial t} = k_{int} C_{\text{rec}}, \quad (6)$$

where C_{MN} is the coated antigen concentration in the microneedle,

$C_{\text{ECM},i}$ is the antigen concentration in the extracellular matrix (ECM) with $i = e$ and d for epidermis and dermis, respectively,

C_{rec} is the antigen concentration bound with the receptors,

C_{cells} is the antigen concentration taken up by the antigen presenting cells,

C_{circ} is the antigen concentration taken up by microcirculation near by the dermis,

$R_{\text{tot},i}$ is the initial receptor concentration,

D_j is the diffusion coefficient with $j = MN, e$ and d representing the coated microneedle, epidermis and dermis, respectively,

k_k is a rate constant describing the velocity of the processes with $k = a, d, c,$ and $int.$, representing the association, and the dissociation rate of the antigen-receptor binding , the rate of uptake into the microcirculation, and the rate of antigen-receptor complex internalization, respectively.

Table 2 The parameter values describing the vaccine transport and skin kinetics

Parameter	Default value
k_a	$1\text{x}10^{5}$[$M^{-1}s^{-1}$]
k_d	$1\text{x}10^{-3}$ [s^{-1}]
k_c	$1\text{x}10^{-5}$ [s^{-1}]
k_{int}	$1\text{x}10^{-3}$ [s^{-1}]
d_{mn}	$0.65\text{x}10^{-8}$ [μmol]
$R_{tot,e}$	$2.2\text{x}10^{-7}$ [μmol/mm^3]
$R_{tot,d}$	$1.0\text{x}10^{-8}$ [μmol/mm^3]
D_{mn}	$8\text{x}10^{-6}$ [mm^2/s]
D_e	$8\text{x}10^{-6}$ [mm^2/s]
D_d	$21\text{x}10^{-6}$ [mm^2/s]

Initial and Boundary Conditions

At the starting time, the antigen concentration is C_0 in the coated region and is zero elsewhere. The initial concentration C_0 is calculated using the initial antigen dose d_{mn} per coated volume. During

the simulation, the antigen concentration at the bottom plane of the skin is fixed to zero, representing that the antigen is taken up into the larger blood vessels. Furthermore, the top plane is impermeable to antigen because it represents the outermost skin layer. The side planes are also impermeable to antigen due to the symmetry of the adjacent skin element volume. The model was simulated until the steady state is obtained, which is when the rates of change in equations (1) – (6) are less than $1x10^{-15}$ μmol per second.

Microneedle efficiency calculation

The efficiency of the immune response was determined from the proportion of the total number of antigens taken up by the cells and the number of activated APCs in the epidermis and dermis. According to the previous study (Römgens et al., 2016), the activation was assumed to depend on their level of saturation, L_{sat}. This parameter is determined by the ratio of the concentration internalized by the cells at the steady state, C_{cells}, and the total receptor, $R_{tot,i}$, of the specific layer of the skin. Hence, the saturation threshold is in the range of 0-1. In this study, if any mesh volume reaches the saturation threshold at 0.5, all cells in that mesh element volume will be activated. The efficiency of the microneedle is obtained from the ratio of APC activation (%), which is the percentage of the activated skin volume per total skin volume, and the antigen amount.

Results

Progression of antigen concentration

The distributions of the free antigen and the internalized antigen as the time evolves are presented in Figure 3. The initial high antigen concentration coated around the microneedle decreases as the antigens diffuse into the skin. Immediately after the antigens release from the coated microneedle, the antigens bind to the APC receptors and are taken up by the APCs. Hence, the concentration of the antigen taken up by the APCs increases over time until it reaches the steady state while the free antigen concentration approaches zero.

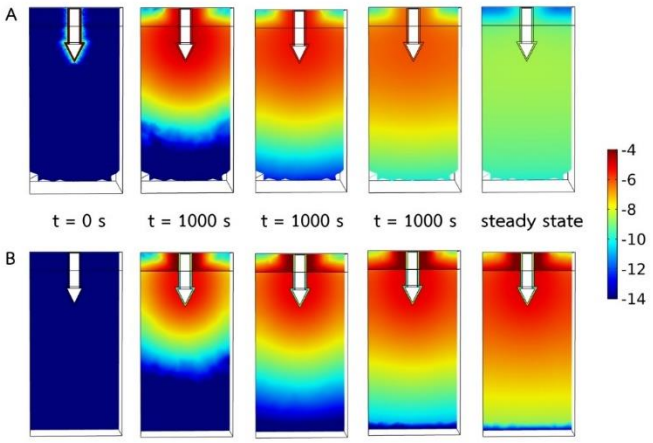

Figure 3 Cross-section snapshots of the progressions of the antigen concentration. Color bar represents the values of a logarithm of the following: (A) free antigen concentration, and (B) the antigen concentration internalized by cells, as the time evolves

Effects of microneedle shape on the APC activation

To assess the effect of microneedle shape, the initially coated antigen concentration was kept the same for each microneedle (1.26×10^{-6} μmol/mm^3). It was found that the shapes of the microneedle clearly affect the APC activation, which is directly related to the activated volume of the skin (Figure 4). The total percentage of the activated volume is the highest for the rectangle with a sharp tip, followed by the cylinder with a sharp tip, the square cone, the circular cone, the arrowhead, and the triangular cone, respectively, as did the activation of the APCs in the dermis. By contrast, in the epidermis the triangular cone can activate the APCs higher than the arrowhead in the epidermis.

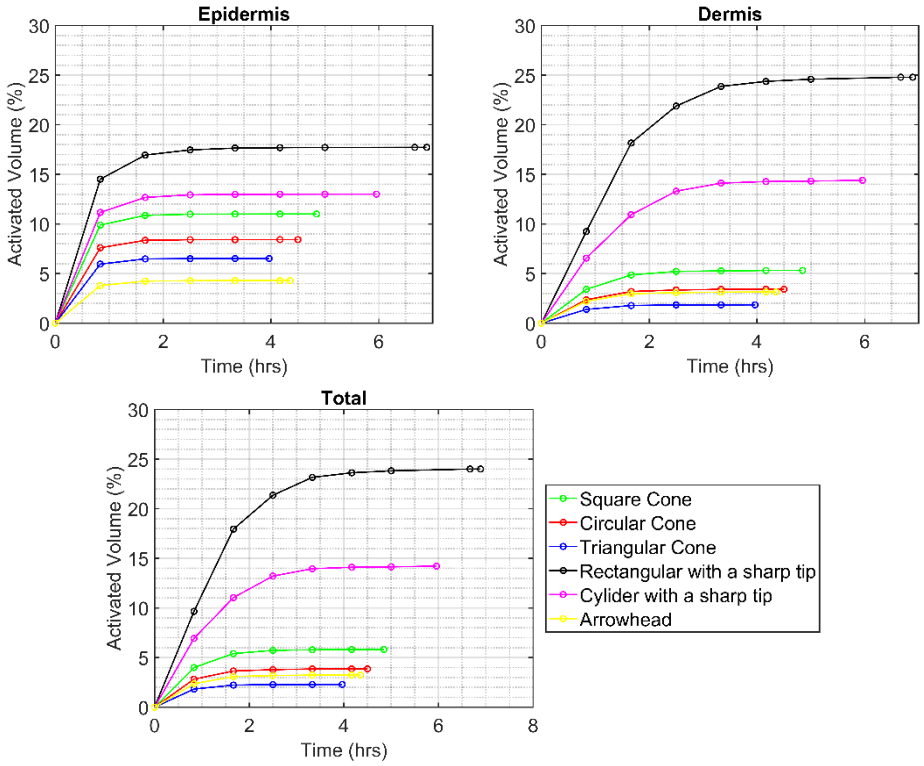

Figure 4 The activated skin volumes as a function of time; the epidermis, the dermis, and the total skin, investigated using the same antigen concentration in each model.

To further evaluate the efficiency of microneedle, the ratio of the total activated volume (%) and the antigen amount coated on the microneedle surface (μmol) was determined. As can been seen in Figure 5A, the efficiency of microneedle is the highest for the rectangle with a sharp tip, followed by the cylinder with a sharp tip, the square cone, the arrowhead, the circular cone, and the triangular cone, respectively. The efficiency of the microneedle is ranging from 0.46 – 1.65 (%/μmol), which infers that the antigen amount of 1 μmol can activate 0.46 – 1.65 % of the total skin volume.

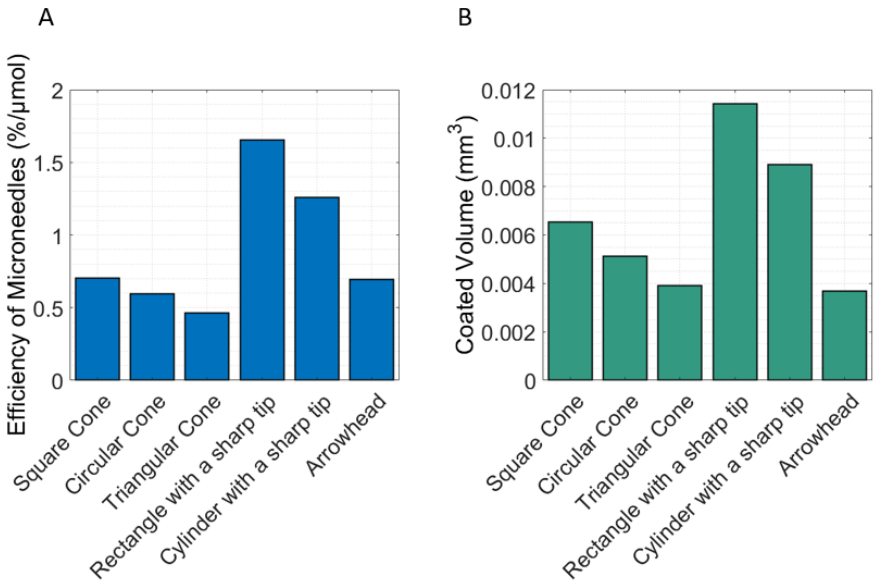

Figure 5 (A) Efficiency of the microneedles calculated by the percentage of activated volume per antigen amount. (B) Coated volumes of the antigen on the microneedle surfaces.

Discussion and Conclusions

Discussion

Although various shapes of the coated microneedle have been designed and fabricated in laboratories with the aim of finding the optimum geometry that is most efficient in activating APCs, there are only a few shapes that are currently used as commercial products (Mackenzie, 2018; Phoosae, 2018). For the vaccine delivery propose, the microneedle must be effective in the skin layers, which have a high number of APCs that are robust for inducing immune responses. The present work assessed the influence of six microneedle shapes that are quite easy to be fabricated in a laboratory (Johnson et al., 2016; Kavaldzhiev et al., 2017; Samant et al., 2018) on the efficiency of the activation of the APCs. According to the previous experimental observation which reported that the evoked immune response was dependent on the area in which the antigens exposed to the APCs (Widera et al., 2006; van der Maaden et al., 2014), in this study, we therefore assumed that the number of activated APCs is directly proportional to the activated volume. We found that the shapes of microneedle affect the total number of the activated APCs and the number of the activated APCs in each skin layer. This is partly due to the structural asymmetry along the vertical axis of the microneedles (Figure 1). With the same antigen concentration, a larger coated volume can store more antigen. Therefore, with these geometries, the rectangular with a sharp tip microneedle provides the highest total number of activated APCs, followed by the cylindrical with a sharp tip, square conical, circular conical, arrowhead, and the triangular conical microneedles, respectively, as did the number of the activated APCs in the dermis. In contrast, in the epidermis the triangular conical microneedle can activate the APCs higher than the arrowhead microneedle. This is due to the fact that the geometry of the triangular cone has a relatively larger

surface area and thus the higher coated volume in the epidermis. Therefore, the triangular cone presents a larger amount of the antigens to the APCs in the epidermis.

The efficiency of the microneedles was further investigated. With the fact that the amount of antigen is directly proportional to the coated volume (Figure 5B), the results turn out that the rectangular with a sharp tip microneedle provides the highest efficiency, followed by the cylindrical with a sharp tip, the square conical, the arrowhead, the circular conical, and the triangular conical microneedles, respectively. The geometry of the six microneedle models can be classified into two types, which are a conical, and a shaft type. As its name suggests, the square cone, the circular cone, and the triangular cone are the conical type. As its geometry shows, the rectangle with a sharp tip, the cylinder with a sharp tip, and the arrowhead are the shaft type. The conical microneedle type has a larger coated volume embedded in the epidermis than the shaft type. This leads to a higher amount of antigen released into the epidermis. The number of activated cells obtained from the conical type in the epidermis was consequently higher than that of the shaft type. In contrast, the higher coated volume of the shaft microneedle type was embedded in the dermis, the higher antigen amount released into the dermis. The number of activated cells obtained from the shaft type in the dermis was thereby higher than that of the conical type. When the dermis has a relatively higher volume than the epidermis, the activated cells in the dermis will dominate over the activated cells in the epidermis.

Conclusion

The present work investigated the influence of the six microneedle shapes on the efficiency in inducing the immune response, which is related to the number of the activated APCs, using the three-dimensional finite element model describing the antigen release from a microneedle and the skin kinetics. It was found that the efficiency of the microneedle-mediated vaccine delivery is highest for the rectangular with a sharp tip microneedle, followed by the cylindrical with a sharp tip, the square conical, the arrowhead, the circular conical, and the triangular conical microneedles. With the fact that the rectangular with a sharp tip microneedle has the largest surface area, it can hold the most substantial amount of the coated antigens. As the amount of the activated APCs relies on the amount of the delivery antigens, this microneedle shape provides the highest efficiency of the vaccine delivery.

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