**Bella-muTM microneedle for dermal penetration of outer membrane vesicle-based vaccine: Proof of concept in human skin explant model**

**Manon Beaujean**1, Jeroen Langereis2, 3, David Boccara1,5, Daniel Dam4, Angèle Soria1,3,6, Gert Veldhuis4, Lucille Adam1, Olivia Bonduelle1, Eric Pedruzzi1, Jeroen Wissink4, Marien Dejonge,2 and Behazine Combadière1

1 Sorbonne Université, Inserm U1135, Centre d’Immunologie et des Maladies Infectieuses (Cimi-Paris),Paris, France;

2 Radboud UMC, Nijmegen, Netherlands;

3 Radboud Center for Infectious Diseases, Nijmegen, Netherlands;

4 U-Needle, De Veldmaat, Netherlands;

5Hôpital Saint Louis, Reconstructive and Cosmetic and Burn, Paris, France*;*

6 AP-HP

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**Footnotes**

MB and JL contributed equally to this work.

MD and BC are senior co-authors of this work.

**Running title:** Microneedle depth and vaccine uptake by skin cells

**Corresponding author information:**

Behazine Combadière, PhD, Centre d’Immunologie et des Maladies Infectieuses (Cimi-Paris), 91 Boulevard de l’Hôpital, Faculté de Médecine Sorbonne université, Pitié-Salpêtrière, 75013 Paris, France, Phone: +33 1 40 77 98 88, e-mail: behazine.combadiere@inserm.fr

﻿**Author contributions:**

MB, BC, & JL, Conceptualization; MB, BC, & JL, Data collection; MB & JL, Data analysis; BC, MD, & JW, Funding acquisition; MB, JL & BC, Investigation; AS & DB, clinical investigators; MB, BC, OB, EP, & LA, Methodology; BC, GV, MD, & DD, Project administration and resources; BC, MD, & JW, Resources; BC, Supervision and validation; MB, JL, BC, & GV, Visualization; MB & BC, Writing the original draft; all authors reviewed and edited the manuscript.

**Abstract (237)**

For intradermal (i.d.) immunization, microneedle delivery systems have been proposed as an alternative to the Mantoux method. However, the penetration depth of microneedles in human skin and the involvement of skin cells are not yet fully analyzed. A new microneedle (Bella-muTM, U-Needle) has a hexagonal shape and silicon material with an ease of use that aims to reduce pain during injection. The ultrashort needle length (1.4 mm to 1.8 mm) allows a perpendicular injection using disposable syringes. We aimed to characterize Bella-muTM microneedles combined with outer membrane vesicle (OMV)-based vaccine using an *ex vivo* human explant model obtained after plastic surgery from healthy donors. We compared 2 sizes of Bella-muTM microneedles (1.4 mm and 1.8 mm) and the conventional Mantoux method to investigate the depth of compound penetration and the skin antigen-presenting cell (APC) capacity to uptake the OMVs. The 1.4 mm Bella-muTM needle deposited the antigen closer to the epidermis than did the 1.8 mm needle or the Mantoux method. Consequently, activation of epidermal Langerhans cells was significantly higher as measured by shortening of their dendrites. We found that 5 subsets of dermal APCs can uptake OMV vaccine whatever the device. In conclusion, i.d. delivery using the 1.4 mm Bella-muTM device combined with OMV-based vaccine allowed epidermal and dermal APC targeting, with major activation of Langerhans cells vs the Mantoux method, showing the accuracy of the microneedle for dermal deposit of vaccine compounds in human skin.

Key words: intradermal (i.d.), microneedle, outer membrane vesicle (OMV), proof of concept, skin antigen-presenting cells (APC).

*Word count:*

**Introduction**

The immunologic environment of the skin makes it a promising route for vaccination because this tissue is rich in professional antigen-presenting cells (APCs), allowing a high quality and intensity of immune response with a low dose of antigen delivered [1]. Strategies to improve vaccine administration into the skin have focused on development of suitable, safe, and efficient devices such as microneedles, patches, and new delivery systems to induce the most effective immune responses. Intradermal (i.d.) vaccination has already proved its strong efficiency with eradication of smallpox virus in 1980 [2]. A major contribution to this achievement was the development of the bifurcated needle by Benjamin A. Rubin [3]. Today, the standard i.d. injection uses the Mantoux method, introduced by Charles Mantoux for the diagnosis of tuberculosis in 1910, and it is currently used for BCG and rabies vaccines [4,5]. The Mantoux method uses a hypodermic 27-gauge needle parallel to and penetrating the stretched skin at an angle of 15° with the bevel upward. The injection is a success if a papule appears [6].

The Mantoux method is not the first choice in vaccination strategies, however, because of its disadvantages and side effects: skin reactions, pain during injection, difficulties to proceed, technical training required for injection, and fluid waste including leakage and dead volume [8,9]. Study results demonstrated a similar seropositivity rate using one-fifth of the intramuscular dose [9], and i.d. vaccination has shown its superiority in intensity and quality of induced immune responses [1]. The difference in the intensity and quality of immune response between the intramuscular and i.d. routes of immunization has been explained by poor variety and quantity of APCs in the muscle compared with the skin [1,10,11]. Since development of the Mantoux method, various techniques and devices have emerged with the aim of high-quality penetration of compounds in the dermis or deposit on the dermis [10].

Although development and manufacturing of microneedle technologies have grown substantially, an understanding of the role of skin APCs has helped in the selection of devices for efficient immunization. Benefits have been demonstrated by targeting specific APCs in the skin: Langerhans cells (LCs; CD1a+ and CD207+) [12,13] in the epidermal layer and macrophage cells (CD163+), and dermal dendritic cells (DDCs; CD1c+) in the dermis [14]. It has been shown that *in vitro* targeting of different APCs elicits multiple immune responses: CD4 T cells, CD8 T cells, and humoral responses [15,16]. Notably, we have demonstrated that targeting of epidermal LCs favours cellular CD8 T-cell responses while DDCs are directed toward humoral responses after vaccination [13,17,18]. One of the techniques using hair follicles, called the cyanoacrylate skin surface stripping, has been developed by our team; this technique has been validated in human skin explants and in a clinical study with different vaccine models (nanoparticles, viral vector, proteins, and DNA). Otherwise, miniaturization of needles has emerged with the development of microinjections or microneedles for the epidermal and dermal penetrations. For example, patches using microneedles (50 µm and 1000 µm long) provide a bridge to transport high-weighted molecules through deep skin layers. A large variety of devices exist (eg, solid, hollow microneedle) [19]. Larger needles (1000 to 2000 µm) have been developed to reach the dermal layer; they are inserted perpendicularly to the skin, making i.d. vaccination intuitive and easy to perform, in contrast to the Mantoux method [7]. Few microneedles pass preclinical development because of the manufacturing processes, which are not appropriate to molecule properties [20,21]. A skin delivery device that has been successfully tested in a phase 4 clinical trial is SoluviaTM (Becton Dickinson) for influenza vaccination [22,23]. However, the cost, shape, and material are not optimal to reduce pain and improve ease of use [23]. Thus, there is still a need to find i.d. devices that are suitable for vaccine delivery; efficiently target skin immune cells; and are usable with various vaccines, easy to use, and acceptable to the patient population.

A new, easy-to-use delivery device, Bella-muTM (U-Needle), with a hexagonal shape and silicon material, aims to reduce injection-related pain. The needle length, which ranges from 1.4 mm to 1.8 mm, allows perpendicular injection using disposable syringes. As an example of vaccine system delivery, we used an outer membrane vesicle (OMV)-based vaccine that protects the antigen from degradation [24]. It has an intrinsic adjuvant activity by the presence of various pathogen recognition receptors ligand (lipopolysaccharide and immunogenic surface protein) targeting innate immune responses [25]. In this study, we used an *ex vivo* human skin explant model to examine the depth of the Bella-muTM device and the efficacy of APC uptake and activation of the OMV-based vaccine.The fresh human skin explants from plastic surgery provide the best support to preclinical experiments of human skin vaccination because they gather more of the human skin microenvironment [26] and have more human characteristics compared with other skin models (animal or artificial) [27]. We compared the 1.8 mm and 1.4 mm microneedles combined with OMV against the Mantoux method. The Bella-muTM device used for vaccination penetrates deeply into the dermis, activates LC of the epidermis, and the OMV vaccine is efficiently taken up by APC after injection in the skin.