Wearable flexible nano-transfection device for on-skin gene editing with CRISPR-Cas9

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Recent advances in *CRISPR-Cas9* techniques have shown possibilities for the 'in-body', patient-specific *in vivo* gene editing, transcriptional modulation and live-cell imaging[1]. However, currently available gene delivery techniques face two major hurdles for direct on-skin transfection of *CRISPR-Cas9*. First, the transfection efficiency is extremely low (< 10 %) for large molecular weights (> 6 kbps, e.g. RNA-guided CRISPR-associated Cas9 protein). Furthermore, targeting to local cells is challenging, impossible for dynamic tracking and analysis. Both issues eventually lead to its difficulty in clinical trials[2].

In this work, we report a novel flexible intracellular delivery nano-device which can be patched on the skin for precise gene delivery into epidermal cells. The device was fabricated on polyimide substrate, which could be easily adjusted for the curves of different skins (**Fig. 1a**). The wearable device integrated multiple functions on one chip. The core of gene delivery is achieved upon nano-electroporation, which consists of array of micro-electrode (width: 20 μm) in connection with nano-wells (diameter: 600 nm) where the *CRISPR-Cas9* is placed for delivery. A micro-needle inserted into the skin is treated as the top electrode. Patching on the skin, the electric field is applied between the top electrode and bottom micro-electrode for cell electroporation (**Fig. 1b**). The nano-well feature could accurately tune the electric field on the cell membrane, which greatly reduces the area affected from the electroporation while improving the cell safety. Uniquely, the concentrated electric field via nano-well 'electrophoretically' drives the charged-cargo (i.e. *CRISPR* and *Cas9* proteins) into cells with high speed (**Fig. 1c**) [3], which achieves precise dose control and high transfection efficiency. The power for cell electroporation is supplied by a wireless communication zone constituted with an ultra-thin magnetic spiral antenna and an near-field communication (NFC) chip. The spiral antenna is made by gold (Au) (thickness: 20 nm) deposition in photolithographic patterning (**Fig. 1d**). NFC technology, remotely controlled by a cell phone, is applied to wirelessly control the cell electroporation. A microfluidic channel (SU-8 photoresist, 50 μm in thickness) is fabricated to connect each nano-well as shown in **Fig. 2a**. *CRISPR-Cas9* flowing in each insulated microfluidic channel to avoid cross-talking. An impedance sensor is also manufactured on the device for testing the connection between the device and the skin. The device was fabricated following the protocol illustrated in **Fig. 2b**.

Preliminary cargo delivery experiments have demonstrated the nano-device can precisely control the dose of *CRISPR-Cas9* delivered into the cells, while maintaining high transfection efficiency and cell viability. At single cell, dynamic tracking gene editing can be realized on the nano-chip. This simple implement to flexible gene transfection nano-device, based on cleanroom nano-fabrication techniques, provide a capability of direct, deterministic *in vivo* gene editing into epidermal cells on the skin.

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[1] Knight, S.C. *et al.* 2015. ***Science***, 350, 823.
[2] Gallego-Perez. *et al.* 2017, ***Nature Nanotechnology***, 12, 974.
[3] Chang L. et al. 2016, ***Nanoscale***, 8, 243.

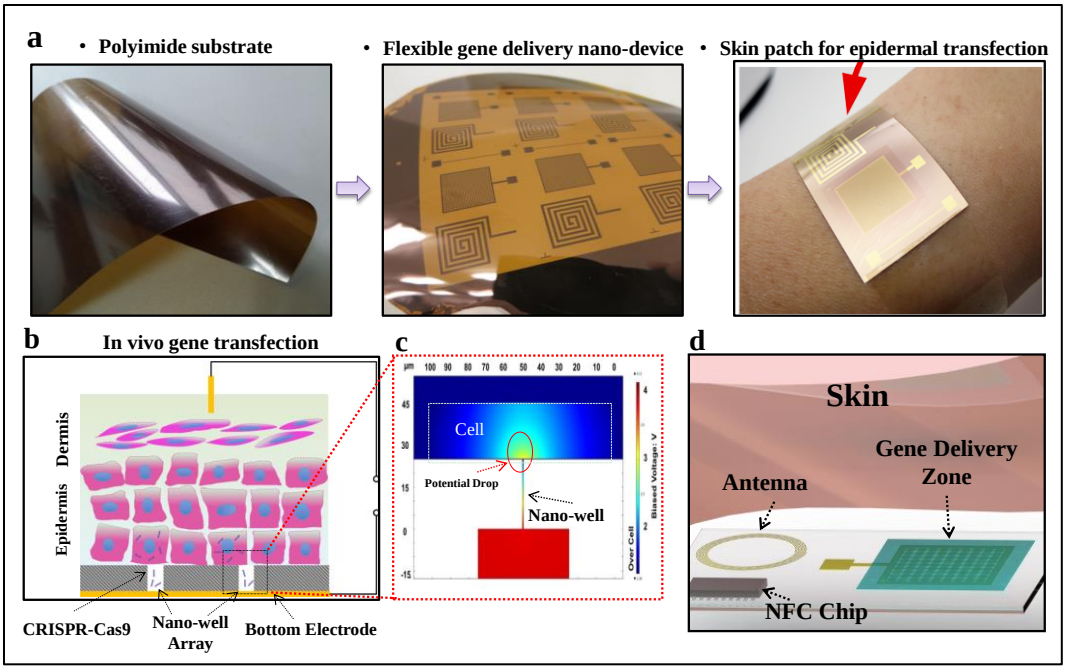

Fig. 1 A flexible intracellular delivery nano-device patched on the skin for precise gene delivery into epidermal cells. (a) The nano-features were patterned on polyimide substrate, which could be easily adjusted to various skins. (b) The schematic illustrates the nano-well array connect the epidermal cells. The electric field is applied between bottom micro-electrode array (bottom electrode) and a top electrode (micro-needle) for electroporating cells. (c) numerical simulation shows the nano-well can concentrate the electric field within a nanoscale-region on the cell membrane, providing a safe potential drop (less than 5 V) for electroporation. (d) The electroporation-assisted gene delivery is powered by wireless communication zone (i.e. a spiral antenna and a NFC chip).

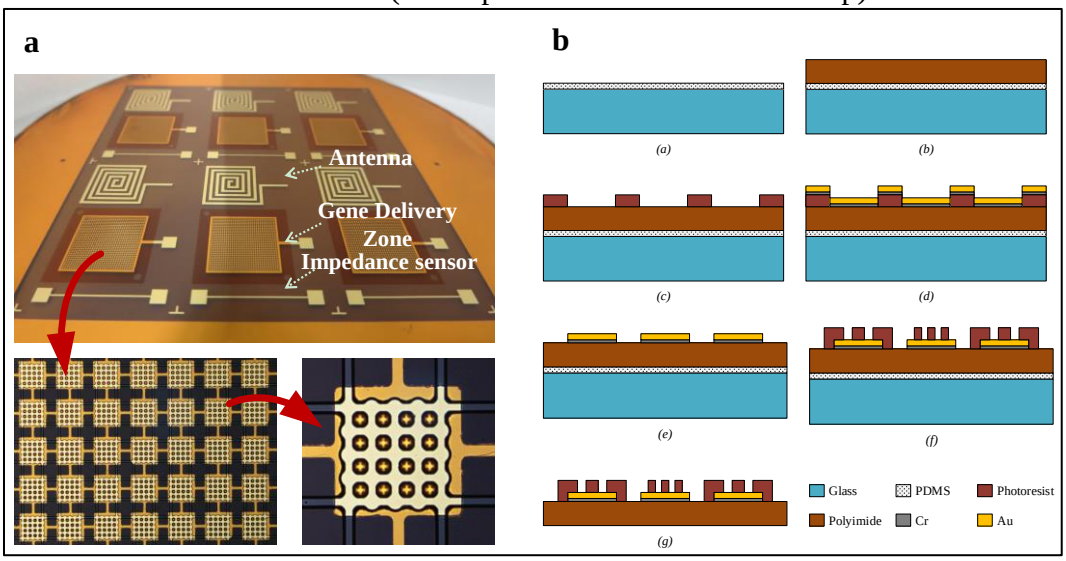

Fig. 2 The photograph of the spiral antenna, microfluidic channel and impedance sensors on the polyimide substrate. (a) Microfluidic channel patterned on the device to connect nano-well array for cargo flow-in and -out. (b) The fabrication protocol of the device.