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HIGHLY ENERGY-EFFICIENT NANO-SPIKE ELECTRIC PROTEIN EXTRACTION (NS-EPEx) CHIPS FOR EUKARYOTIC CELLS

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ABSTRACT

In this paper, we present a nano-spike electric protein extraction (NS-EPEx) chip for highly energy-efficient protein extraction from mammalian cells. 3D periodic Nano-spikes (NSs) arrays with controllable dimensions were fabricated on low-cost Aluminum (Al) foils using scalable nano-imprinting, electrochemical anodization and etching processes. Extracted protein yield was comparable to one obtained by chemical method. By properly tuning pulsed electric field, proteins can be extracted either by keeping cells alive or lysed. For the same protein yield, NS-EPEx chip can be operated at applied voltages of 4V and save energy by 2~3 orders of magnitude (0.12kJ/kg) as compared to the other methods (10~1,000 kJ/kg).

KEYWORDS: Nano-spike electric protein extraction chip, Pulsed electric field, Energy efficient

INTRODUCTION

Biopharmaceutical products, such as human growth hormone, vaccines, monoclonal antibodies, recombinant proteins are usually manufactured in eukaryotic cells, fungus or bacteria. Downstream processing, i.e., separation, purification, etc., is the major factor in production costs. Process efficiency can be improved by selectively releasing intracellular products in primary recovery phase, i.e., cell disruption [1]. Optimization of cell disruption to avoid complete cell disruption and micronization of cell debris is very important from economic viewpoint [2]. Conventional methods (bead milling, homogenization, chemical, enzymatic) affect protein stability by disintegration of vacuoles releasing proteases [2]. Pulsed electric field (PEF) has been proved effective in avoiding these drawbacks by selectively disrupting the cell membrane in fast and rapid manner (*ca* milliseconds) [2]. Cellular analysis using small amounts of intracellular proteins over time with minimal invasiveness is possible with PEF. Conventional and PEF extraction methods are limited due to their high energy requirements even at the laboratory scale; special power generators (few tens of kV) are required to fulfill such requirements [3]. Low-energy PEF extraction methods are highly desirable in commercial applications as well as in Lab-on-a-chips.

EXPERIMENTAL PROCEDURE

In this paper, we present a nano-spike electric protein extraction (NS-EPEx) chip for highly energy-efficient protein extraction from mammalian cells (Fig. 1a). 3D periodic Nano-spikes (NSs) arrays with controllable dimensions were fabricated on low-cost Aluminum (Al) foils using scalable nano-imprinting, electrochemical anodization and etching processes (Fig. 1b). Complete details of fabrication of nano-spike chips can be found elsewhere [4-5]. Electric pulses with adjustable amplitude V_a and duration t_P were generated using DAQ card and Labview program (Fig. 1a). HeLa cell suspension was exposed to PEF on NS-EPEx chips and the extracted protein concentration was determined on supernatants after centrifugation by standard Bradford protein assay (Fig. 1c). Numerical simulations of the NS array using COMSOL show the nano-focused electric field due to the local electric field enhancement near the NSs (Fig. 2). Low input energy was required to extract proteins on NS-EPEx chip due to nano-focused electric field near the NSs.

RESULTS AND DISCUSSION

Extracted protein yield increases with cell concentration (Fig. 3) and cell concentration of 5×10^6

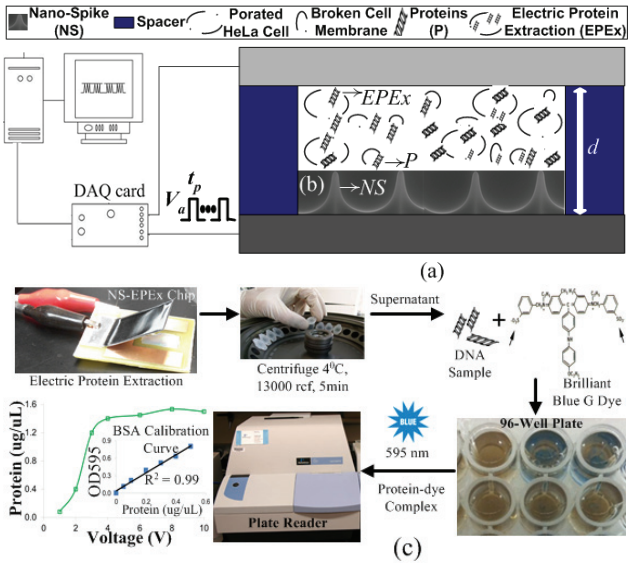

Figure 1. (a) Schematic of a NS-EPEx chip and the experimental setup. The applied electric field was focused and enhanced through Nano-spikes (NS) on the NS-EPEx, (b) SEM micrograph of array of Aluminum NS and (c) standard Bradford protein assay after electric protein extraction.

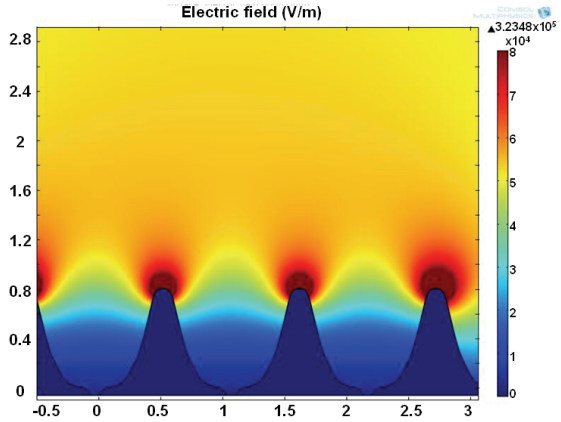

Figure 2: Numerical simulation showing the electric field distribution on the nano-spike array using COMSOL at V_a = 4V shows the nano-focused electric field due to the local electric field enhancement near the NS.

cells/mL was used in further PEF experiments. PEF parameters V_a and t_P have significant impact on extracted protein yield which increased by increasing V_a and t_P (Fig. 4). For t_P =10ms and V_a ≥4V, protein concentration was comparable to one obtained by chemical method (Fig. 4). By properly tuning PEF parameters, proteins can be extracted either by keeping cells alive or by cell lysis (Figs. 1 & 4).

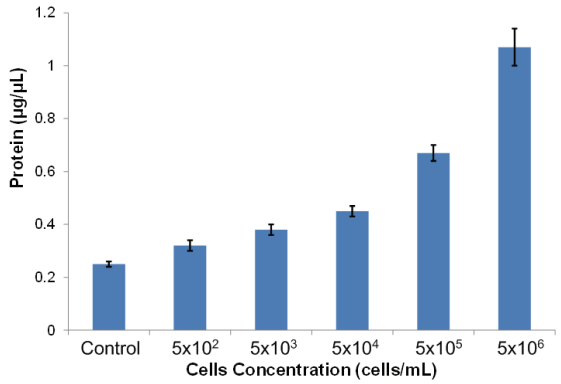

Figure 3. The protein yield of the NS-EPEx chip as a function of HeLa cell concentration at Va=4V (E_a=3.5kV/cm) and t_P=4ms. Each graph represents the average of 3 measurements and the error bars represent standard deviation.

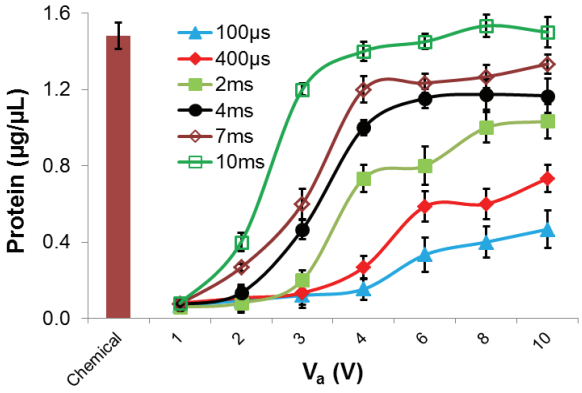

Figure 4: The protein yield of the NS-EPEx chip as a function of applied voltage V_a at selected pulse duration t_P at the cell concentration of 5×10^6 cells/mL.

From the protein yield–V_a curves (Fig. 4), two critical values of voltage/electric field was defined. $E_{c,ex}$ is the electric field for the onset of protein extraction; beyond this value, protein extraction efficiency increased exponentially. $E_{s,ex}$ is the critical electric field for the saturation of protein yield. Phase diagram for the protein extraction from HeLa cells on the NS-EPEx chip were constructed using these criti-

cal values (Fig. 5) which defines the effective protein extraction area with PEF parameters. Typical energy requirement for the protein extraction using different extraction methods was compared with our method (Table 1). It is clear that for the same protein yield, NS-EPEx chip can save energy by 2~3 orders of magnitude (0.12 kJ/kg) as compared to the other methods (10~1,000kJ/kg).

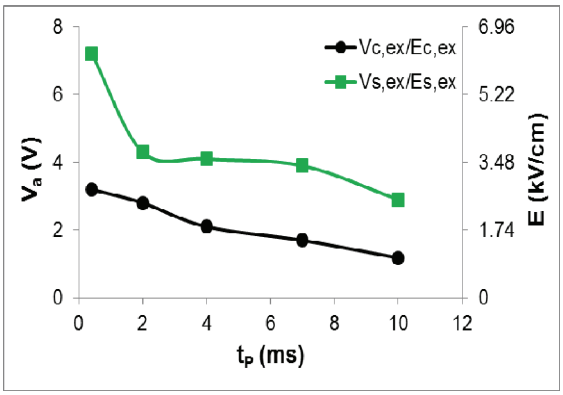

Figure 5. Phase diagram for the protein extraction from HeLa cells on the NS-EPEx chip. $V_{c,ex}/E_{c,ex}$ is the critical voltage/electric field for the onset of protein extraction; beyond this value, protein extraction efficiency increased exponentially. $V_{s,ex}/E_{s,ex}$ is the critical voltage/electric field for the saturation of protein yield.

Table 1. Typical energy requirement for the protein extraction using different methods.

Ref.	Extraction Methods	Specific Energy Requirement	Protein Yield %
[6]	High pressure homogenization	150–1500 kJ/kg	91%
[6]	Ultrasonication	12–96 kJ/kg	1.8%
[6]	High voltage electrical discharge	53.1 kJ/kg	1.15%
[6-7]	Pulsed electric field	13.3-53.1 kJ/kg	5.2% (2mg/mL)
This paper	**NS-EPEx**	**0.12 kJ/kg**	**90~100% (1.5µg/µL)**

CONCLUSION

In this paper, we present a nano-spike electric protein extraction (NS-EPEx) chip for highly energy-efficient protein extraction from mammalian cells. Extracted protein yield was comparable to one obtained by chemical method. By properly tuning pulsed electric field, proteins can be extracted either by keeping cells alive or lysed. For the same protein yield, NS-EPEx chip can save energy by 2~3 orders of magnitude (0.12 kJ/kg) as compared to other methods (10~1,000kJ/kg).

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