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電子・情報工学専攻	学籍番号	063321	
申請者 氏名	牛流章弘		

指導 教員	石田誠 澤田和明 河野剛士

論　文　要　旨　(博士)

論文題目	ナノスケール先鋭化プローブアレイの形成技術と そのデバイス応用に関する研究

(要旨　1，200 字程度)

細胞への“外来遺伝子導入”により遺伝子発現量を変化させ細胞の挙動を解析する手法は，生理学の分野において非常に重要な実験ツールある．近年では細胞集団中に存在する特定の細胞に対して活性生体分子 (DNA，RNA，機能タンパク質) を局所的に導入し，細胞集団における DNA 導入された細胞の影響・効果，さらに活性生体分子の機能を確かめたいという要望がある．この要望に対する技術的課題は，細胞へのダメージを最小限にしつつ，ある特定の細胞内に活性生体分子を導入する，局所的な活性生体分子の導入法である．そこで、この技術的課題を克服するため新たな”局所的”遺伝子導入手法として，マイクロマシニング技術による silicon nanowire (SiNW) や carbon nanotube (CNT) などに代表されるナノワイヤを利用した手法が提案されている．しかし，ナノワイヤによる DNA 導入手法はその主な対象が培養細胞のような in-vitro の環境下であり，in-vivo 環境下のような生体深部への DNA 導入を目的とした，例えば 100μm 以上の長さを有する高アスペクト比のナノワイヤは，ワイヤの強度の観点から実現困難である．そのため細胞刺入用プローブの長さは長いものでも 10μm 程度にとどまっている．このように現状では in-vivo での局所的遺伝子導入は未だ困難であり，生体深部への刺入を可能とするナノプローブの実現が求められている．本研究では，選択的 Vapor Liquid Solid (VLS) 結晶成長による高アスペクト比長針 Si プローブによるナノスケール先鋭化 Si プローブアレイの開発を行い，その応用として生体刺入型局所 DNA 導入・ナノインジェクターデバイスや，細胞刺入が可能となるナノプローブの利点を生かした細胞内電位計測用神経電極アレイへの実用化に向けての取り組みを行った．ナノスケール先鋭化プローブアレイ(以下ナノチッププローブ)の実現に向け，選択的 VLS 結晶成長技術による Si プローブの先端の選択的加工法を提案し，直径 2μm，プローブ長さ 30μm－200μm の VLS-Si プローブに対して，細胞へのプローブ刺入が可能となる先端直径 100nm ナノスケールに先鋭化した Si プローブの製作に成功した．ナノチッププローブによる局所的 DNA 導入法の確立に向け，培養細胞用：プローブ長 25μm，脳スライス用：100μm，200μm，プローブ間隔 100μm，20×20 アレイのそれぞれプローブ長の異なる 3 種類のナノチッププローブを製作し，遺伝子導入実験を試みた．ナノチッププローブによる DNA 導入デバイスへの応用を実証する為，HEK293 やヒト皮膚繊維芽細胞，従来手法では困難であった厚さを有する脳スライス(厚さ 400μm)の生体サンプル対してプラスミド DNA の導入実験を行い，電気的に DNA を吸着さしたナノチッププローブの細胞刺入を行うことで、それぞれ培養細胞サンプルよりプラスミド DNA を発現させることに成功した．微小の部位に対して低侵襲かつ局所的 DNA 導入を行えるという新たなコンセプトの DNA 導入法を実証した本研究成果は，先鋭化ナノスケール Si プローブによる生体刺入型デバイスとしての可能性を示すものである。

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Department	Department of electronic and information engineering	ID	063321
Name	Akihiro Goryu		

Supervisor	Makoto Ishida Kazuaki Sawada Takeshi Kawano

Abstract

Title	Fabrication and applications of nanoscale tipped microwire arrays

(800 words)

Nanoscale devices show their great potential as a measurement technology with a high spatial resolution for nanoscale biological experiments. In particular, vertically aligned nanowire devices have enabled numerous biological experiments, including intracellular nanowire penetration, DNA/molecule transfer, optical imaging, and nanoscale transistor penetration into a cell. To form nanodevices with a higher aspect ratio, millimeter length semiconducting nanowires and carbon nanotubes have been synthesized (e.g., silicon nanowires by vapor–liquid–solid (VLS) growth and carbon nanotubes by a water-assisted synthesis). However, the deep penetration of such nanowires/tubes within a thick biological sample has not been demonstrated because nanowires/tubes bend or break with a penetration depth of several tens of microns, preventing the nanowires/tubes from reaching the target tissue or cells. Thus, an obstacle for nanoscale device applications in biological experiments is deep wire penetration, which will enable three-dimensional (3D), multi-site, nanoscale measurements in thick biological samples. To realize a penetrating nanodevice array, we have proposed employing a vertically aligned VLS grown silicon microwire scaffold array as a mechanical support. In this approach, chemical etching of silicon wires sharpens the tip section on the nanoscale (<100 nm in diameter at the tip). The fabrication process developed in this paper facilitates nanoscale tipped microwire electrode array devices, and the results suggest that the nanowire array has applications for both particle trapping and particle depth injections, also DNA transfer to the cultured cells and brain tissue. Advantages of the fabrication process include low-voltage electrical trapping over a wide area and batch particle manipulation with a constant wire interval.

We start with a silicon (111) substrate consisting of silicon microwire arrays assembled by a selective VLS growth. The diameters of the as-grown silicon wires were 2–4 μm, and all of the wires had a constant length of 30 μm. The proposed nanotip formation process is as follows. First, the wires are spray-coated conformally with a photoresist. Second, the tips of the wires are selectively exposed from the photoresist by plasma etching with O_2 + CF_4. Third, the tips are wet etched with a HF + HNO_3 solution at a constant temperature of 40°C (etching time = 1–3 min). Finally, the photoresist is removed with acetone. Silicon microwire arrays can be formed to create sharpened nanotips with a tip diameter of less than 100 nm by utilizing the batch-processed silicon chemical etching, and also the tip angles are achieved ranging from 11° to 38°. The chemical etching based nanoscale sharpening process is repeatable, fast, batch-processable and IC-compatible. In addition, the sharpening process using photoresist spray-coating and plasma etching without additional masking is theoretically a height-independent process. In principle, it could be used for samples with even higher aspect ratios, such as silicon microwires more than 100 μm long, promising simple fabrication of a variety of 3D micro-or nano-devices.

To demonstrate nanoinjector by nanotip wires, herein 3D nanoscale tipped electrodes with various lengths were fabricated, based on the sharpening process for VLS grown silicon microwires. After coating the nanotip silicon-wire with gold, the wire was encapsulated with a parylene layer, and only the nanotip section can be exposed from the parylene by photoresist spray coating and subsequent photoresist etchings. As a promising device application, we demonstrate the trapping of polystyrene nanoparticles in a solution using a fabricated gold-nanotip wire array. The nanotip wire with a 150 nm curvature radius and a 4.2 μm^2 electrode area exhibited a locally enhanced trapping performance with a low trapping voltage of 20 mV. Moreover, these trapped nanoparticles can be injected into a soft material (gelatin), demonstrating a multi-site wide-area batch depth injection and an assembly of nanoparticles. Such nanotip wire arrays should be applicable to trap numerous particles, including DNA/molecules attached to gold particles, and may realize injection into biological tissues and individual cells/neurons.

For a new class of the gene transfers, multipoint local DNA transfers into individual cell were demonstrated by a nanotip wire array. Gene transfer techniques including gene gun and AFM wire are very powerful tools in physiological sciences, however, further technical requirements such as multipoint/batch and deep area transfer techniques are still problematic. Here, we showed multipoint/batch and localized DNA transfers into individual human embryonic kidney cells (HEK293) with plasmid containing yellow fluorescent protein (YFP). After pressing the nanotip wire array into the cells, individually DNA transferred cells were observed. Such nanotip wire-based DNA transfers are very simple process, but this promises fast and reproducible DNA transfers. Additionally, deep area DNA transfers into thick samples such as brain slices can be possible by using the nanotip wire arrays, promising a powerful experimental tool for numerous biological experiments.