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TOYOHASHI
UNIVERSITY OF TECHNOLOGY

PRESS RELEASE

Source: Toyohashi University of Technology, Japan, Committee for Public Relations

Release Title: DNA stamper injections using nanoscale-tipped wire arrays
Release Subtitle: A convenient and powerful tool in neuronal research

Overview:
Toyohashi University of Technology research team led by Prof. Rika Numano and Prof. Takeshi Kawano showed DNA stamper injections can be performed delivering biomolecules into live neuronal cells within brain tissues *ex vivo* and *in vivo* using nanoscale-tipped wire (NTW) arrays to genetically modify and restore cell function in the brain.
These NTW array injections followed the exploration of pacemaker cells in the brain slice of central clock with knockdown function using short-hairpin RNA injection of an indispensable clock gene in circadian rhythms.

Details:
The NTW arrays with diameters <100 nm and wire lengths of approximately 200 µm are fabricated through the growth of silicon microwire arrays and nanotip formation, manufactured originally at the Electronics-Inspired Interdisciplinary Research Institute (EIIRIS) at Toyohashi University of Technology. They show that DNA can be delivered in a minimally invasive manner and expressed in multiple cells in a brain slice *ex vivo* and in a living mouse brain *in vivo* by simply pressing the NTW array for genetic recombination like pressing a stamp onto a surface of brain tissue (Fig.1).

This technique uses pinpoint multiple-cell DNA injections into deep areas of brain tissue at ease, enabling target cells to be marked by fluorescent protein expressional vectors (Fig.2). Moreover, the nanowire-based injection of biomolecules indicates that it is a convenient and powerful tool for genetically altering the function of living brain cells, including the injection of short hairpin RNA (shRNA) for the knockdown functions and gRNA and CAS9 vector DNA for genome editing.

In this study, DNA stamper injections of shRNA of *Bmal1*, a critical clock gene in mammalian circadian rhythms with about a 24 hr period, repress the function of pacemaker cells in the suprachiasmatic nucleus (SCN) brain slice of *Per1*::*luc* transgenic (Tg) mice (Fig.3).
This technique has potential for not only biological transfection into neuronal cells, but also for electrophysiological recordings. Our NTW array injection technique enables genes for marking and the functional modification of living cells in deep brain tissue areas during intracellular recording. It is helpful for overcoming the bottleneck in the neurophysiological study.

Development Background:

The Electronics-Inspired Interdisciplinary Research Institute (EIIRIS) at Toyohashi University of Technology has the LSI factory for designing, processing, and evaluating LSIs, sensors, and MEMS. It is equipped with a life science experiment facility that conducts animal and bio-experiments. Therefore, at EIIRIS, the developed device is applied in physiological experiments, and the results are immediately fed back for the improvement of the device, and research teams from different scientific fields collaborate on technology development and research. The layout of multiple wires loaded on the chip can be freely changed for the gene stamp using an NTW array device, so that it can be updated to a more suitable one through feedback depending on the target site.

Future Outlook:

The intracellular potential can also be measured with the NTW array after device interconnection, so finally, they can identify which cells are measured by the NTW array with a fluorescent signal after the fluorescent marker DNA is inserted into the cells during electrophysiological measurement. In addition, since this gene stamping technology can be used for knockdown such as RNAi and for knockout, genome editing and gene manipulation, it is possible to genetically manipulate a specific part of the brain. The research team hopes to contribute utilizing gene stamping technology for new research to elucidate brain functions in the field of neuroscience.

Funding agency:

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Reference:

Rika Numano, Akihiro Goryu, Yoshihiro Kubota, Hirohito Sawahata, Shota Yamagiwa, Minako Matsuo, Tadahiro Iimura, Hajime Tei, Makoto Ishida, and Takeshi Kawano. Nanoscale-tipped wire array injections transfer DNA directly into brain cells *ex vivo* and *in vivo,* FEBS Open Bio, 2022 *in press,* doi:10.1002/2211-5463.13377.

Glossary:

gRNA (guide RNA): A short RNA strand with a scaffold sequence necessary for binding CAS9 protein to determine the cleavage site of CAS9 for genome editing by CRISPR- CAS9 systems.

shRNA (Short hairpin RNA): A short RNA strand with a hairpin structure for silencing target gene expression by RNAi (RNA interference).

Per1::*luc* transgenic mouse: A mouse in which the *Per1*::*luc* recombinant gene is inserted into the genome of all cells to monitor the circadian rhythms using bioluminescence. *Per1* is one of the clock genes with rhythmic expression with a 24 hr period. *Per1*::*luc* is a recombinant gene in which the *Per1* promoter region is followed by the luciferase enzyme derived from the firefly as a reporter.

scRNA (scrambled RNA): A short RNA strand with a random base sequence was used as a negative control to the functional shRNA.

Further information

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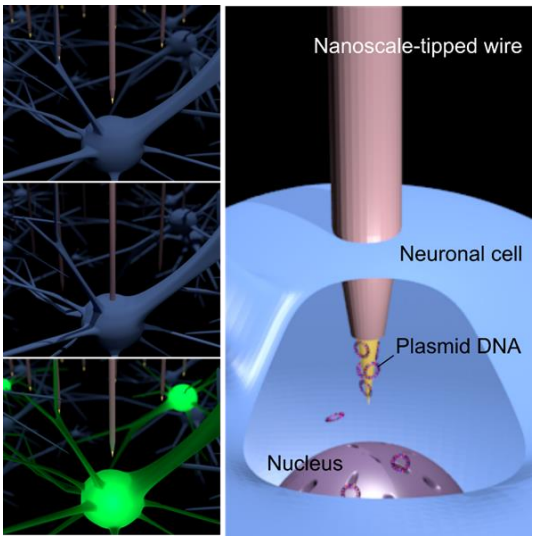
Website: https://www.tut.ac.jp/english/

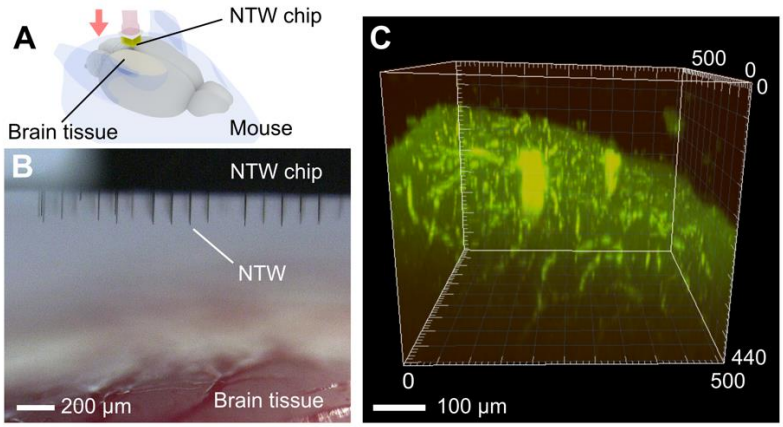
Figure1:



Title: DNA stamper injections using NTW arrays

Caption: DNA can be delivered and expressed in multiple cells in brain tissue *ex vivo* and *in vivo* by simply pressing the long nanowire array to express a fluorescent protein.

Figure2:



Title: *In vivo* injection of Venus plasmid DNA into the brain tissue of a living mouse using a 200-µm-long NTW array.

Caption: (A) Diagram showing the *in vivo* injection of Venus plasmid DNA into a mouse's cortex using an NTW array. The NTW array, which was held in place by a micromanipulator, penetrated the tissue's barrel area through a window in the skull. (B) A photograph showing the NTW chip positioned over the barrel area of the cortical surface. (C) Three-dimensional images of the Venus fluorescent signal in the whole brain after the injection.

Keywords: Neurons, Gene transfer, Short hairpin RNA, Brain tissue, Transfer RNA, Genetic technology