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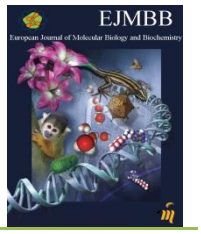

NANOBIOPSY: AN EMERGING INNOVATIVE TOOL FOR INTERROGATING LIVING CELLS

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ABSTRACT

Several approaches have been employed over the years to study tissue architecture and cellular heterogeneity. These have provided insights into cell to cell interaction and circuitry within tissues. Biopsy is one of them and is considered to be the gold standard for definitive diagnosis. Since this procedures are performed on tissues which are removed either from living (biopsy) or dead individuals (autopsy), our ability to study the dynamic function of individual cells in heterogeneous populations in living state has reduced. Thus there is a need to develop a technique which would perform biopsy on tissues & cells without killing them. Robotic nano biopsy has been developed by researchers which is a nano technology based tool to sample living cells without killing them. It uses a glass nano pipette of 50-100 nm in diameter to pierce the cell membrane to extract a volume of 1% of cell and is based on a customized Scanning Ion Conductance Microscope. The tip is so fine that it causes minimal cell destruction. Researchers have used this technique to extract and sequence RNA from individual human cancer cells and mitochondria from human fibroblasts and sequence the mitochondrial DNA. It can also be used to deliver material into cells, opening up ways to re-programme diseased cells. It is a versatile platform for cancer biologists and stem cell biologists who are trying to understand what is happening inside the cell.

INTRODUCTION

Several approaches have been employed over the years to study tissue architecture & cellular heterogeneity. These have provided insights into cell to cell interaction and circuitry within tissues. Biopsy is one of them and is considered to be the gold standard for definitive diagnosis. Since this procedures are performed on tissues which are removed either from living (biopsy) or dead individuals (autopsy), our ability to study the dynamic function of individual cells in heterogeneous populations in living state has reduced. Thus there is a need to develop a technique which would perform biopsy on tissues & cells without killing them.

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Science is undergoing yet another change in helping mankind enter a new era, the era of nanotechnology. "Nano" is derived from the Greek word for 'dwarf'. Nano technology is manipulating matter at nanometer level roughly the size of 2 or 3 atoms [1]. In simple words, 1 nm = one-billionth or 10^{-9} of 1 meter. Nano biopsy is an emerging field in which culturing of a cell is not only observed by its mass behaviour, but can also be observed on individual cellular level. Single cell injections can also allow direct manipulation on the individual cellular level [2].

Manipulation and analysis of individual cells is the key in understanding processes that control single-cell behaviour in complex environment. Nano technology-based tools having high sensitivity and low invasiveness are holding great promises as new biomedical devices for single cell manipulation [3]. Nano biopsy is the latest

method that enables extraction of tiny amounts of cellular material from living cells, approximately 50 femtoliter (fL), which corresponds to approximately 1% of the volume of a cell. The major advantage of nano biopsy over other systems is that this system can extract tiny samples from inside a living cell without killing it because the pore of the nano pipette is so small that the insertion is minimally invasive. Also biopsy can be taken from the same cell multiple times over couple of days as the cell recovers within 5 seconds post-aspiration [4].

METHODS

Robotic glass nano pipette with a diameter of 50-100 nm is the major tool required. (figure1) .

The fully electrical read-out as well as the ease and low cost of fabrication are unique features those give nano pipette technology enormous potential. After using nano pipettes as a biosensor, now it is being used for single-cell manipulation. Researchers have developed a single-cell manipulation platform based on quartz nano pipettes. The system uses scanning microscopy techniques to position the nano pipette with nano scale precision. The nano pipette is fitted with electrodes to mediate voltage-dependent injection or aspiration form individual cell [3]. The nano pipette is integrated into a customized Scanning Ion Conductance Microscope (SICM) [4]. Scanning ion conductance microscopy is a scanning probe technique that has recently gained attention for its ability to image living cells with high spatial and temporal resolution. SICM relies on a glass nano pipette filled with an electrolyte solution biased to generate an ion flow through the nano pipette aperture [5, 6]. The nano pipette is then scanned over a surface and changes in the magnitude of the measured ion current reflect the topography of the sample [7,8]. The SICM system uses an ion current across the tip of the nano pipette as a feedback signal. Custom-designed software directs the nano pipette towards the cell until it detects a 0.5% drop in ionic current. At this point, the software stops the approach & quickly lowers the nano pipette by 1 micro-meter at high speed (100 micrometer/sec).This pierces the cell membrane, inserting the nano pipette tip into the cell membrane.

When a nano pipette is filled with an organic solution & immersed into an aqueous one, a liquid-liquid interface is formed at the nano pipette opening. If a voltage is then applied across this interface, a force is generated that can induce the aqueous solution to flow into/out of the nano pipette [9]. The nano pipette contains a solution of 1, 2-dichlorethane (DCE) containing 10 mM tetrahexyl ammonium tetrakis- (4-chlorophenyl) borate (THATPBCl) that creates an interface on contacting the cell's cytoplasm [4]. This electro wetting mechanism is used in the nano biopsy procedure.

The application of voltage across the interface brings about the changes based on the voltage. Negative voltage causes the cytoplasm to flow in the nano pipette (influx) while the positive voltage causes the solution to flow out from the nano pipette (efflux). After piercing the cell membrane, the voltage is switched to – 500 mV for 5 seconds. This causes controlled influx of cell cytoplasm into the nano pipette. Then the bias is switched to 100 mV, which stops the influx but does not cause efflux of the aspirated contents. The nano pipette is then quickly raised. The aspirated content is transferred into a 5 micro-meter droplet of RNase-free H_2O by application of +1 V for 2 minutes and kept at 4^o c (figure2). Overall, out of the 50+ nano biopsies performed by Paolo Actis et al during their study, they obtained 70% success rate.

Fig 1. Nano pipette tip (Image courtesy- mrc.org.ua)

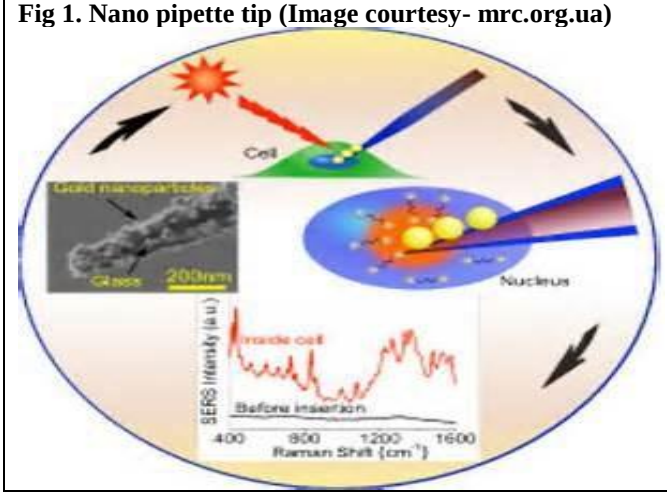
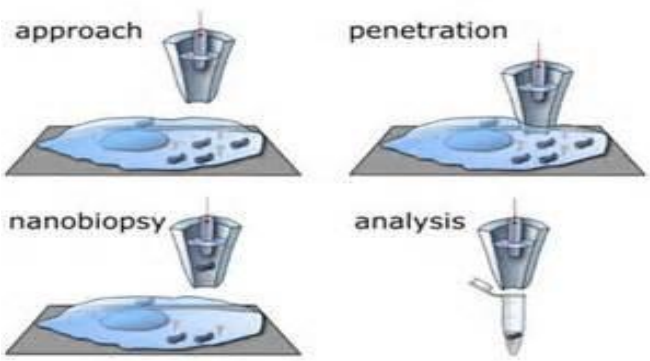


Fig 2. Schematic of single cell nano biopsy (Image courtesy- www.kurzweilai.net)



RESULTS AND DISCUSSION

Nano biopsy can be used to extract and sequence RNA from cancer cell to reveal how tumorigenesis-related signaling pathways can cause genetic changes which ultimately lead to the transformation of a healthy cell to a cancerous cell. Current methods for single-cell manipulation often can detect only one class of analytes. In contrast, the nano biopsy platform has sub cellular resolution and can be used to isolate small subpopulations of mitochondria from single living cells, and quantify

mutant mitochondrial genomes in those single cells with high throughput DNA/RNA sequencing technology. Minute amounts of total RNA and mitochondrial DNA were taken out of a single cell and nucleic acids were analyzed using next generation genomic sequencing techniques by Paolo Actis *et al* in Santa Cruz, United States. Nano biopsy can be used as an important tool in oral cancer research and clinical management, role of heterogeneity in primary tumour tissues and systemically identifying critical parameters in disease progression and potential metastatic states. Nano pipette has been recently utilized to measure the electrophysiology at small synaptic buttons, to locally deliver molecules to multiple sub cellular areas, and to trap molecules in lipid bilayers [2].

Nano biopsy can be used to shed light on the importance of mutations in the mitochondrial genome. It is a versatile platform for anyone trying to understand what is happening inside the cell, including cancer biologists, stem cell biologists, and others. This technique can be used to dynamically study how cancer cells are different from healthy cells, or look at how brain cells are affected by Alzheimer's disease. It can also be used to deliver material into cells, opening up ways to re-programme diseased cells. The use of nano pipettes to aspirate just a few copies of mitochondrial DNA from a living cell might provide the basis for less invasive and more accurate monitoring of disease progression. Nano biopsy may also lay the foundation for developing new classes of drugs to attenuate neuro - degeneration or to treat diseases as diverse as Parkinson's and Alzheimer's [4].

CONCLUSION

Nano biopsy is minimally invasive nano surgical technology to interrogate living cells. It can be used as a platform to assess mitochondrial mutation rates, in cancer research & its clinical management. Thus nano biopsy may provide foundation for dynamic sub cellular genomic analysis.

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CONFLICT OF INTEREST: Nil

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