Development of micropatterned biochip microscopic platform for early diagnosis of neurological diseases through exosomal detection of microRNA from liquid biopsies

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Abstract

Biomarkers in extracellular vesicles (EVs) purified from human liquid biopsies have been suggested to hold great clinical values for advanced diagnostics and precision medicine. Recent progress in cancer biology using highly-sensitive fluorescence microscopy (single molecule TIRF) and microfabrication bioengineering in detection of exosomal mRNA elevates the likelihood that the exosomal RNA molecules and their membrane proteins can act as dual biomarkers for diagnostics of pancreatic cancer [4]. We have attempted to apply similar technological pipeline in detection of microRNAs (miRNAs) as biomarker of RCVS (Reversible cerebral vasoconstriction syndrome) and migraine, discovered and reported by TVGH research teams led by Dr SJ Wang and Dr SP Chen; moreover, we aimed to improve the drawbacks in the first generation of biochips by registering normalizers embed in the biochip. High-precision photomasks have been successfully manufactured in the early stage of this research; we then processed the production of biochip using synthetic polymers modified by ultra-low wavelength of UV for micropattern able to trap Lipoplex NanoParticles (LNPs) containing molecular beacons. Pilot study showed that 1) normalizers display an expected linear increase of intensity; 2) standard vesicles can interact with LNPs capable of emitting correct fluorescence signals. These results demonstrate that our novel design of biochip exhibits a better effectiveness to obtain the florescence readouts than that of previous generation. Currently we are using rtPCR to validate whether those miRNAs would be changed in the fraction of EVs from plasma of normal and patient. The LNP-based biochips will be further used to detect exosomal miRNA/mRNA in other neurological diseases, such as Parkinson and Alzheimer. This assay is critical for linking the EV Biology and plausible biomarkers of RNA to meet the unmet clinical needs.

Research Significance

1. The lack of direct and accurate diagnosis methods in neurological diseases often results in a time-consuming and complicated diagnosis process and a higher possibility of misdiagnosis.
2. In our research center, the unique discovery of microRNAs as biomarkers in RCVS (miR-130a-3p, miR-130b-3p, let-7a-5p, let-7b-5p, and let-7f-5p) and migraines (miR-155, miR-126, miR-21, and Let-7g) can be insightful for identifying patients carrying the diseases [1,2].
3. However whether those miRNAs are specific to the diseases and enriched in EVs purified from serum or cerebral spinal fluid are yet to be examined.
4. We aim to develop a precise diagnostic tools for detecting the diseases by monitoring those changes of biomarkers in EVs. The assay we establish could serve in early diagnostics and longitudinal research and reduce the burden of social/health welfares.

Research Goal

1. We will use conventional reverse transcription polymerase chain reaction (RT-PCR) method to examine whether these miRNAs are enriched in the exosome of plasma from normal entity and patients.
2. Improvement of surface chemistry will be implemented to our novel design of biochip, which can increase signal-to-noise ratio for the detection of very weak fluorescence signal given by configuration change when molecular beacons binding to targeting miRNAs.
3. Purified EVs/exosomes from patients will be examined in our biochips and microscopic platform.

Experimental design

Figure 1.

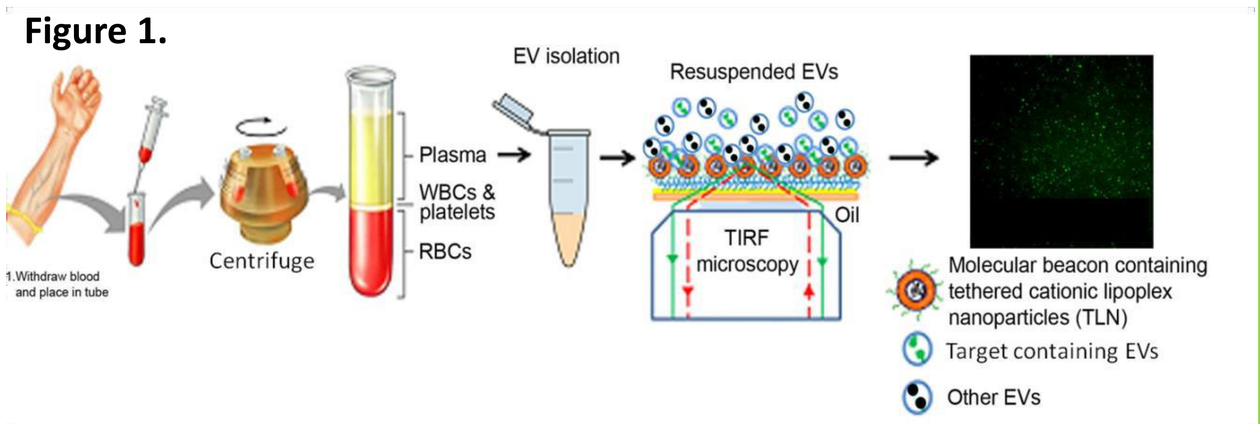


Figure 1. Schematic diagram of tethered lipoplex nanoparticles (TLN) biochip technology: target biomarkers containing extracellular vesicles (EV) are isolated from patient plasma samples and will be captured by TLN (molecular probes containing LNPs that are tethered on the biochip) and emits fluorescent signals. The intensity of the fluorescent signals can be observed and quantified by the total internal reflection fluorescence (TIRF) microscopy [3].

Figure 2.

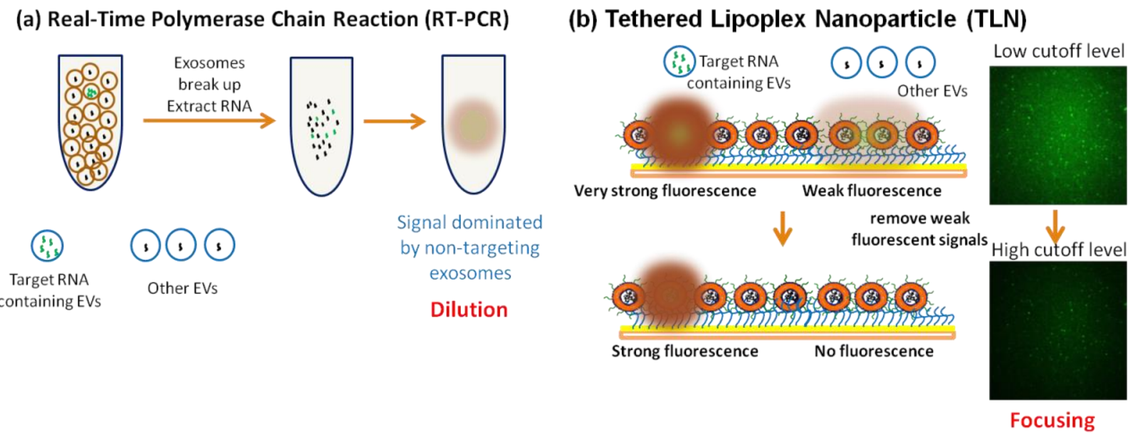
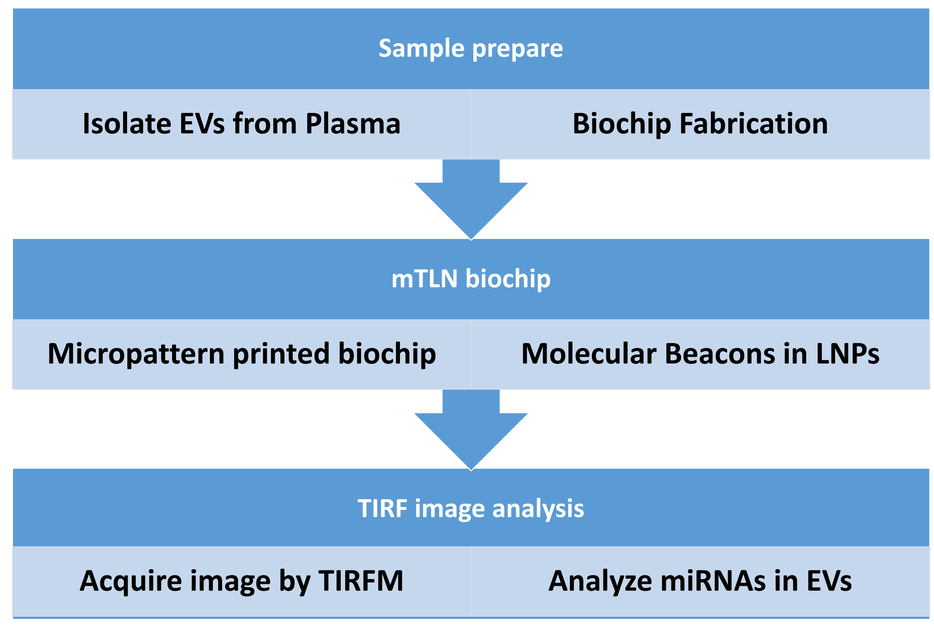


Figure 2. Schematic diagram of the comparison of the (a) traditional real-time polymerase chain reaction (RT-PCR) technique, and (b) our novel TLN technique. The RT-PCR technique will need to break up all the specific and non-specific vesicles result in a “dilution” effect, while the TLN technique can increase the signal cutoff threshold to get a clearer fluorescent signal of the specific bindings result in a “focusing” effect [3].



Result

Figure 3.

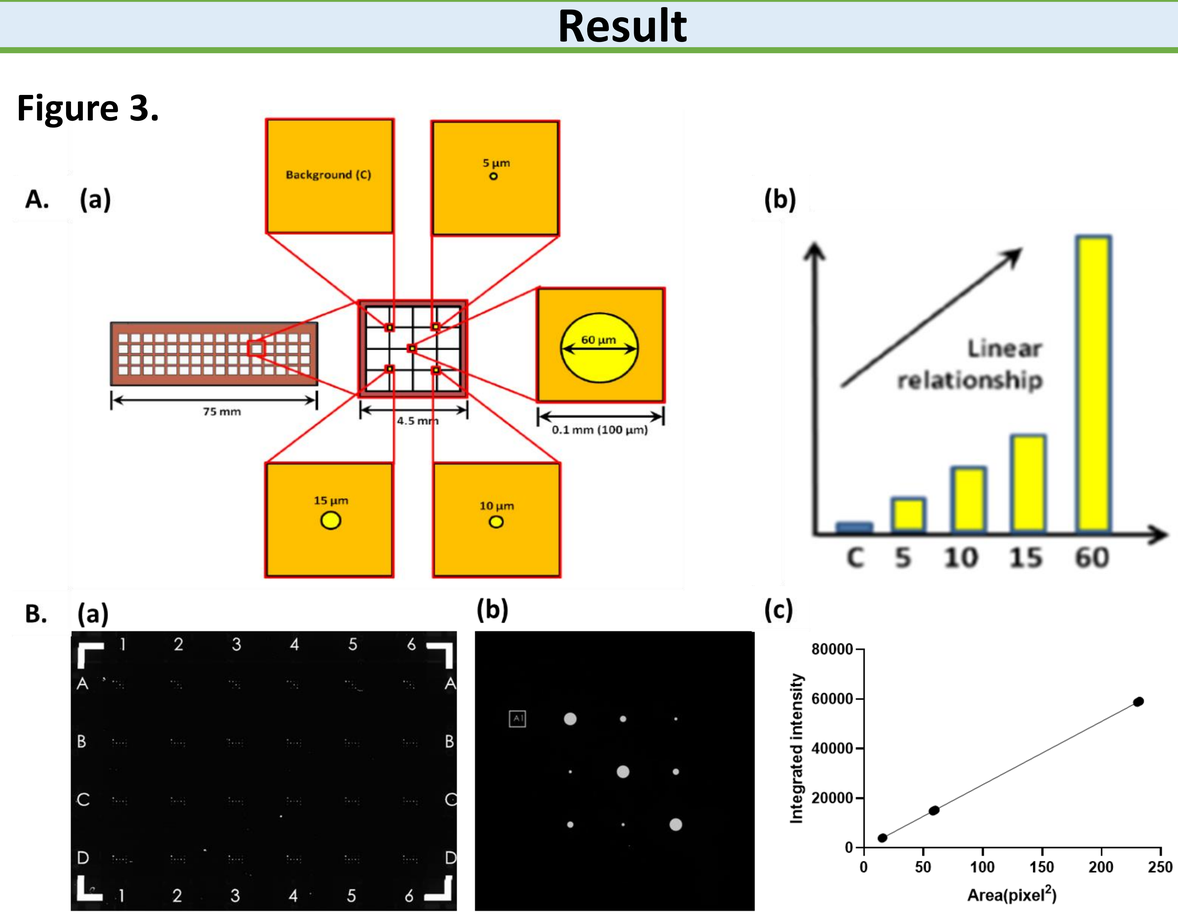


Figure 3. Design of biochip in 64-well format shown in A; 24well in B; a normalizer with different surface area of micropattern are embed on PLL-PEG coated surface(A-a). The theoretical prediction of increased intensity shown in A-b. A real entire photomask of 24 well format is fabricated shown in B-a; In B-b a single well is zoomed in detail; B-c is shown for quantification of intensity of various surface areas.

Figure 4.

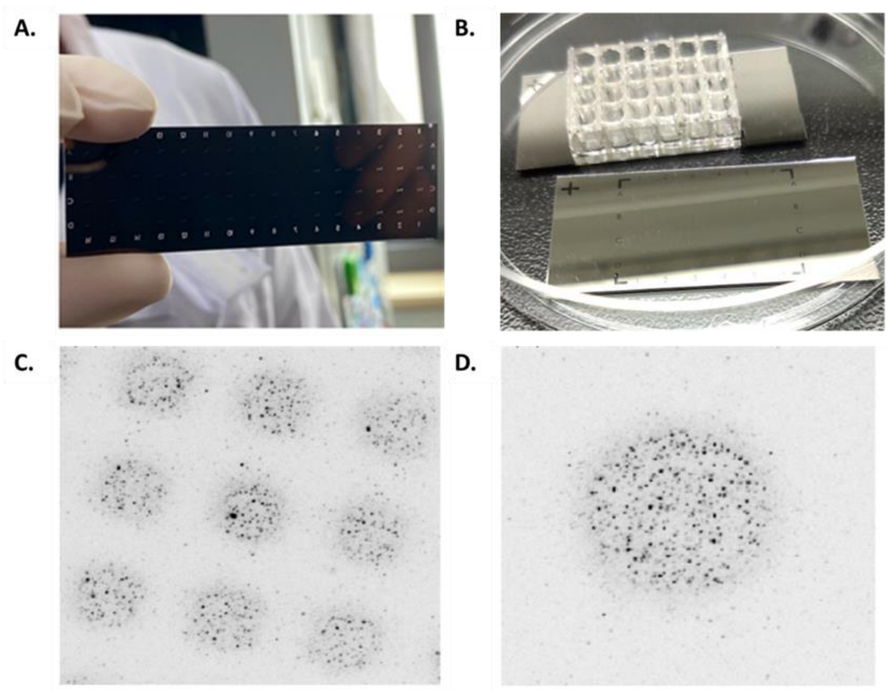


Figure 4. A is a 64 well format of biochip. B is 24 well biochip with compartment made by PDMS. C. reverse contrast of fluorescence signal emitted from the interaction of standard vesicles with LNPs trapped by hydrophilic micropattern (TIRF objective N.A.=1.4) is well arrayed followed by the design of phtomask. D. Reverse contrast images reveal that fluorescence signal from micropattern (imaged by TIRF 100X N.A.=1.49) are more facile to monitor and be quantified. Individual EVs can be observed on the micropattern whereas the neighboring non-patterned areas are in very low florescence intensify.

Reference

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