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OPTICAL DIAGNOSTICS

Nanosensors for liquid biopsies

Carbon nanotubes enable the optical detection of nucleic acids in biofluids.

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Disease onset, progression and response to treatment can in principle be detected or monitored by analysing tumour cells, extracellular vesicles and cell-free nucleic acids circulating in biological fluids, such as blood and urine. Indeed, 'liquid biopsies' offer opportunities for non-invasive screening and diagnostic assays that are also more economical and less demanding in terms of processing time and expertise than conventional procedures. Cell-free nucleic acids, such as circulating DNA, microRNA (miRNA) and other non-coding RNAs, can be used as disease biomarkers (in particular, in oncology, but also in other pathologies and contexts, including myocardial ischaemia, liver damage, multiple sclerosis, and the prenatal screening of various conditions[1–4]). However, although the relationship between circulating tumour DNA (ctDNA) levels and cancer progression was first reported in 1977 (ref. [5]), and the first correlation of a specific miRNA blood profile with the prognosis of a disease (chronic lymphocytic leukaemia) in 2005 (ref. [6]), the lack of standardized, accurate and unbiased oligonucleotide-detection

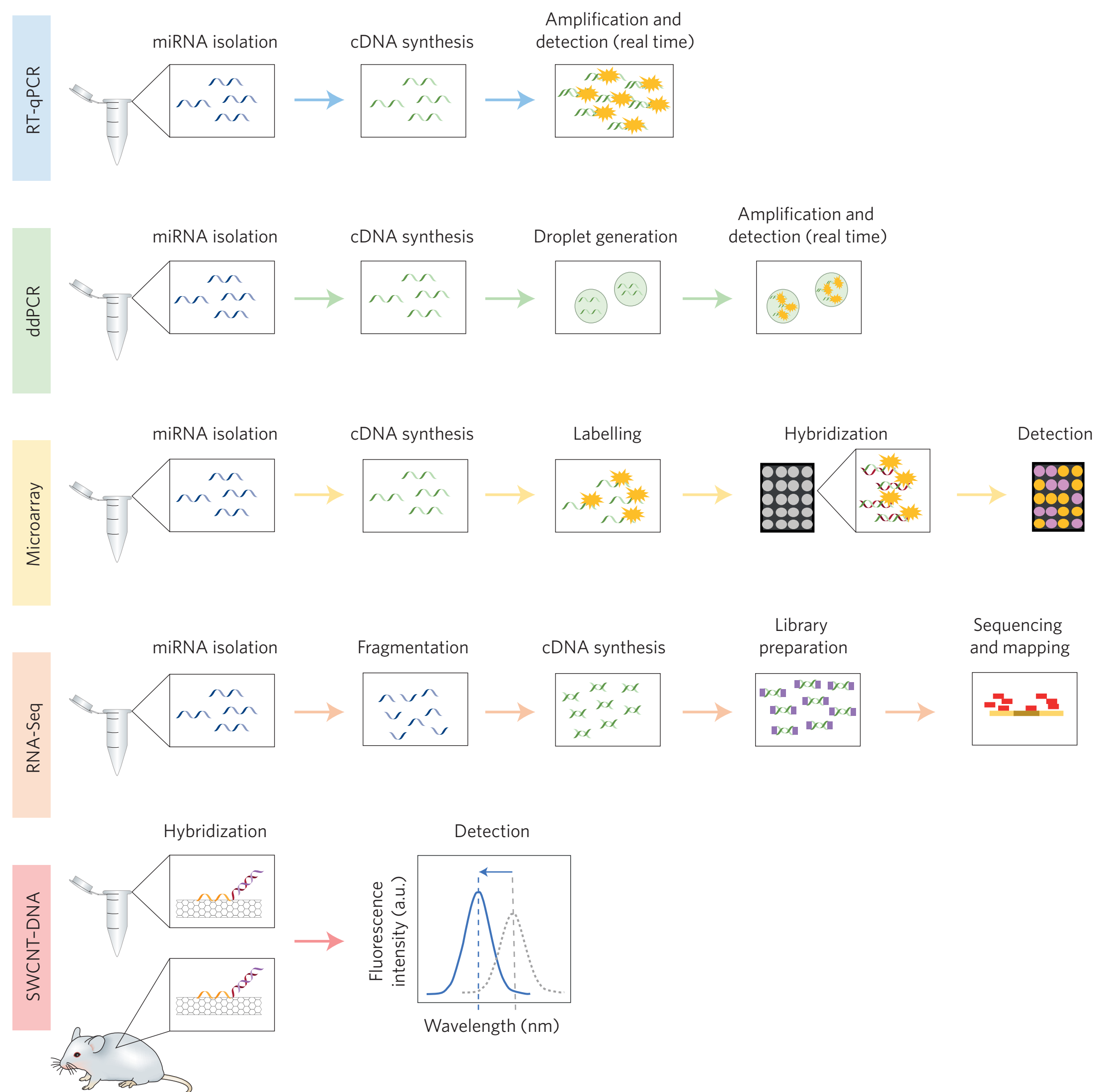

Figure 1 | Workflows for current miRNA-detection technologies. Processing steps required for the detection of specific miRNA sequences in currently established techniques (RT-qPCR, ddPCR, microarray, RNA-Seq) and in the SWCNT–DNA sensor[7].

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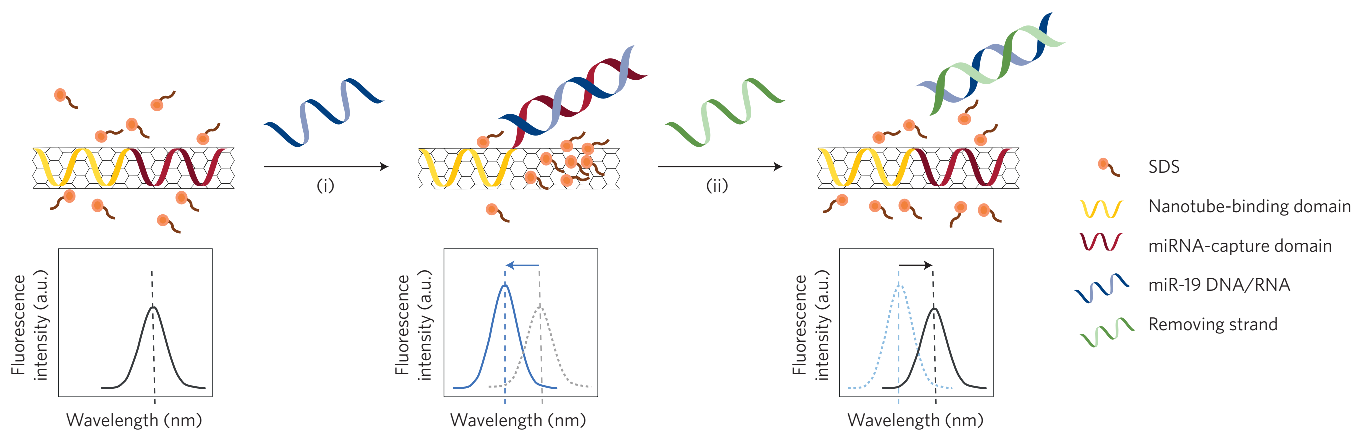

Figure 2 | Mechanism of detection of the SWCNT–DNA sensor. The sensor consists of a SWCNT and a DNA strand composed of a nanotube-binding domain (yellow) and a miR-19-capture sequence (red). The complex emits fluorescence in the near-infrared region. In the presence of miR-19 DNA or RNA (i), the miRNA-capture domain desorbs from the nanotube surface to bind its target, leading to a blue-shift in the emission wavelength and to an increase in fluorescence intensity as the newly available surface is populated by surfactant molecules (sodium dodecyl sulfate, SDS) available in solution. The addition of a removing strand (ii) displaces the hybridized miRNA-capture domain, and resets the spectral properties of the sensor.

technologies that can be translated into point-of-care diagnostics has delayed the adoption of liquid biopsies in clinical settings. Reporting in *Nature Biomedical Engineering*, Daniel Heller and colleagues now describe an optical biosensor that takes advantage of the spectral properties of single-walled carbon nanotubes (SWCNTs) to offer a potentially radical alternative to the currently established nucleic-acid detection methods[7] (Fig. 1).

State-of-the-art methods for the detection of miRNA include reverse-transcription quantitative real-time polymerase chain reaction (RT-qPCR), microarrays, and next-generation sequencing[2]. RT-qPCR, and its more statistically powerful version, droplet digital PCR (ddPCR), offer high sensitivity; however, variability in the efficiency of retro-transcription and of complementary DNA (cDNA) amplification can lead to bias. Hybridization-based techniques such as microarrays are only semi-quantitative and suffer from poor sensitivity. Small RNA sequencing (RNA-Seq) provides an unprecedented wide coverage of targets, but its more complicated workflow and data analysis limit lab-to-lab reproducibility. Also, the need to isolate the nucleic acids of interest from the relevant biological fluid introduces an additional source of variability in all these methods; indeed, different protocols and commercial kits have been shown to favour the enrichment of specific RNA species[8]. Altogether, these technical roadblocks may be to a large extent responsible for the lack of consistency among miRNA–disease profiles reported by independent laboratories[9]. In light of such hurdles, there is a need to either establish specific guidelines that minimize experimental variability and that ensure the reliability of results — such as the MIQE (minimum information for publication of quantitative real-time PCR experiments) guidelines[10] — or to develop technologies that completely bypass the limitations of existing methods.

The biosensor of Heller and colleagues consists of a SWCNT–DNA complex, wherein the nucleic acid sequence contains a nanotube-binding domain, $(GT)_{15}$, and a miRNA-capture domain complementary to miR-19 (a clinically relevant miRNA for its role in oncogenesis). SWCNTs have been used as components for biosensors for years because they respond to molecular interactions on their surface with shifts in their excitation and emission wavelengths and with detectable changes in fluorescence intensity. Resilience to photobleaching and emission in the near-infrared window, which allows for imaging in the biological milieu, are additional advantages. In Heller and co-authors' work, the miRNA-capture domain dissociates from the nanotube surface only in the presence of miR-19 DNA or RNA (but not of other random, length-matched oligonucleotide sequences), hybridizing its target and resulting in a blue shift in the emission and excitation wavelengths, as well as an increase in fluorescence intensity (Fig. 2). By means of fluorophore-quenching experiments, molecular dynamics simulations and thermodynamic analyses, the authors found that the spectral response was affected by a decrease in the density of phosphate ions in the proximity of the nanotube surface, as the hybridized domain remained stably dissociated from the carbon backbone. The specificity of the biosensor is indeed remarkable: not only did it remain unresponsive in the presence of a library of 4^{23} random oligonucleotide sequences, but it was also able to sense miR-19 when included in such a pool. More importantly, the SWCNT-based sensor was able to almost perfectly discriminate between three sequences of the same miRNA family with only a few base mismatches. The capacity to differentiate between such minute differences in an oligonucleotide sequence is vital for high-performance liquid-biopsy analyses, since the difference between a 'normal' and a 'diseased' sequence often involves a very small number of nucleotides.

Heller and co-authors also demonstrated the capacity of the SWCNT–DNA complex to sense synthetic miR-19 added in serum or urine, and after injection into the peritoneal cavity of living mice. These findings reinforce the specificity of the SWCNT–DNA biosensor, in particular because of the abundance, in bodily fluids, of other circulating oligonucleotides and macromolecules that could interfere with the detection of the target. Also, direct detection circumvents the bias introduced by nucleic-acid purification and enrichment steps involved in current technologies, such as RT-qPCR. An additional advantage of the SWCNT–DNA complex is the opportunity to recover its sensing capacity via displacement of the hybridized target, that is, by introducing a removing strand that through competitive binding leads to the re-adsorption of the miRNA-capture domain to the nanotube surface.

Both the direct-detection and signal-reversal possibilities of Heller and colleagues' biosensor suggest that it could make the basis of a wearable sensing device that eventually enables real-time *in situ* measurements. However, most circulating miRNA travels either sequestered in extracellular vesicles,

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or associated to high-density lipoproteins or other stabilizing proteins such as Ago1 and Ago2 (ref. [11]). Therefore, detection of the endogenous nucleic acid in such forms should be demonstrated to unambiguously prove that miRNA isolation is not a requirement for a functioning SWCNT–DNA sensor. In addition, the recovery of sensing capacity would have to be translated to *in vivo* conditions. As currently designed, the SWCNT–DNA sensor needs repetitive administration of a removing strand; also, the kinetics of target displacement and of miRNA-capture-domain re-hybridization to the nanotube surface are significantly slower than the kinetics of the sensing event.

The strength of Heller and colleagues' detection method stands on the capacity of SWCNTs to interact with single-stranded DNA and on their extraordinary photoluminescence. However, this is only one of the several properties of SWCNTs that allow the transformation of a molecular interaction into a detectable signal. For example, changes in Raman scattering have been used to detect ctDNA in blood samples[12]. Other nanoscale materials can also improve the detection of circulating nucleic acids, owing to their large surface-to-volume ratio, which allows for efficient interactions with the given target and therefore grants high sensitivity. Because of the localized surface-plasmon-resonance effect, the leading nanomaterial class for such use is indisputably gold nanoparticles (mostly nanospheres but also nanorods), also in combination with other nanomaterials such as graphene. Gold-based sensing technologies also enable the detection of tumour-circulating cells and extracellular vesicles, as well as the simultaneous detection of cell-free nucleic acids and proteins[13].

It may still be early days to predict if and when novel biosensing technologies — including those based on nanomaterials — that attempt to circumvent rather than solve several of the limitations of current detection methods may develop into routine point-of-care diagnostics. However, Heller and colleagues' biosensor already offers a series of advantages over state-of-the-art oligonucleotide-detection techniques: direct detection that bypasses the need for nucleic-acid isolation; utilization of the nanotube's intrinsic photoluminescence, which renders labelling unnecessary; and high sensor sensitivity, which abolishes the need for target amplification. In addition, the distinct spectral properties of different nanotube chiralities offer opportunities for multiplexing that, although probably not comparable with the coverage of sequencing technologies, could match that of other detection methods such as RT-qPCR. ❐

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