Review Article

Review On Nano Flare: A Novel Diagnostic Probe.

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ABSTRACT
It is important these days to develop the new methods which will provide early detection of cancer to reduce metastatic risk before the existence of secondary tumors. Clinical treatments administered after metastatic tumor diagnosis are not effective. If the cancer is detected at early stage then available therapies can be administered and patient prognoses can be improved. Amongst all the therapies available, Nano flare is a novel diagnostic probe which offer a new method for detecting cancer biomarkers using live cells. This technique is based on the nanotechnology. These probes are based on spherical nucleic acid and are composed of gold nanoparticle core and densely packed with highly oriented oligonucleotide shells; these sequences are complementary to specific mRNA targets and are hybridized to fluorophore labeled reporter strands. When target mRNA binds to capture strand, reporter strand gets displaced and shows fluorescence which can be detected by using any fluorescence detection platform. Nano flares hold promise for the early detection of cancer markers in living cells.

KEYWORDS: Nano flare, Smart flare, Merck Millipore, Spherical Nucleic Acid, Nanoparticles, Diagnostic probes.
1. Introduction
Detection of cancer is a multi-stage and timely process. Occasionally cancer gets detected by chance or screening may be the option. The final diagnosis of cancer is based on the pathologist’s opinion. Various techniques are available to detect cancer. These include Computed Tomography (CT), Magnetic Resonance Imaging, Positron Emission Tomography (PET), Ultra sound examinations, Laboratory tests and cancer markers, Genetic testing, etc. but by using these methods, cancer can be detected at last stages after metastasis.[1] Patients whose cancer has detected early are much more likely to have a positive prognosis and outcome. Numerous techniques like, qRT-PCR and antibody staining, are widely used for cancer detection. It is very important to develop new methods which provide early and accurate assessment of metastatic risk before establishment of secondary tumors so that available therapies can be administered and patient prognoses can be improved. Nano-flares are a new class of intracellular molecular probes. Design of Nano Flare allows to bind genetic targets in cancer cells, and also generate light when that particular genetic target is found. Clinical treatments administered after metastatic tumor diagnosis are often ineffective. Detecting biomarkers is an evolving technique in biological research. Nano-flare Probes offer a new method for detecting biomarkers - using live cells.[2] This technique is based on the nanotechnology. These probes are based on spherical nucleic acid (SNAs) and consist of gold nanoparticle cores and densely packed and highly oriented oligonucleotide shells; these sequences are complementary to specific mRNA targets and are hybridized to fluorophore-labeled reporter strands. The Nano Flare enters the cells via endocytosis, a normal mechanism by which the cell engulfs extracellular material by entrapping them into a bag made of cell membrane.[3] Molecules and particles which enter the cell by endocytosis normally remain trapped in this bag. This entrapping is essential to protect us from viruses and bacteria by preventing them from accessing the cell machinery. The endosome suddenly fades away leaving the particles free to diffuse in the cell and meet their RNA targets.

2. Methods
Nano flare is a diagnostic probe based on nanotechnology for early detection of the cancerous cell. These probes are based on spherical nucleic acid (SNAs) and are typically composed of gold nanoparticle cores and densely packed and highly oriented oligonucleotide shells; these sequences are complementary to specific mRNA targets and are hybridized to fluorophore-labeled reporter strands.[4]

2.1. Structure of Nano flare
Structure of Nano-flare consist of gold nanoparticle with two hybrid oligonucleotide strands. One is capture strand which is directly attached with gold nanoparticle and second is reporter strand which consist of fluorescence to emit light while detection of target mRNA. Reporter strand is quenched with capture strand as shown in figure 1.[3]

2.2. Preparation of Nano flare Probes
- To prepare a mixture add 3nMol of oligonucleotide per 1ml of 10nM gold colloid.
Shake for a period of 12 hours.

- Add sodium dodecyl sulfate solution (10%) to above mixture to achieve 0.1% concentration.
- Then add 0.1M phosphate buffer solution (pH 7.4) to achieve 0.01M phosphate concentration.
- Add six aliquots of sodium chloride solution (2.0M) to the mixture for 8 hours period to achieve sodium chloride concentration of 0.15M.
- Shake overnight to complete functionalization process.
- The solution containing functionalized particles are centrifuged at 13000 rpm for 20 min and resuspended in phosphate buffer saline (PBS); 137nM NaCl, 10nM phosphate, 2.7mM KCl, pH 7.4) three times to produce the purified gold nanoparticles.
- Purified oligonucleotide functionalized Au NPs can be suspended to concentration of 10nM in PBS containing 10nM of complementary Cy5 labeled reporter sequence.
- Heat the mixture to 70°C and cool to room temperature to hybridize the reporter flare to nanoparticles.
- Resulting particles can be stored in dark place for at least 12 hours to allow the hybridization.
- Then particles can filter and sterilized by using 0.2 micrometer (µm) acetate syringe filter.
- Oligonucleotides can be synthesized by using an automated oligonucleotide synthesizer (e.g. expedite 8909 nucleotide synthesis system).
- Bases and reagents required are available readily.
- Oligonucleotides obtained can be purified by reverse phase HPLC. \[4\]

2.3. Working Mechanism of Nano flare probe

A capture oligonucleotide is bound to the gold nanoparticles. A reporter strand is bound to the capture strand. The reporter strand carries a fluorophore but it does not emit light because it is too close to the gold (the fluorescence is “quenched”). In the presence of target RNA, the reporter strand is replaced by the target RNA and therefore released, quenching stops, and fluorescence is detected as shown in figure 2. \[5\]

2.4. Steps involved in introduction of Nano flare probe in live cell

2.4.1. Internalization

Internalization is nothing but the entering of probe into the cell. Current method to analyze the content of the cell involves killing of cells, utilizing transfection reagent, electroporation to enter live cells. In other case Nano flare probes enter cells by receptor mediated endocytosis. This is the normal cellular process due to this, there is no need to use any type of forceful mechanism to enter probe into the live cell as shown in figure 3.\[5\][6]

2.4.2. Interrogate target specific mRNA

After entering Nano flare probe into cells, the next step is detecting target mRNA of live cell. Nano flare probe has unique architecture to combine specific target mRNA. It is sequence-based recognition. Nano flare consist of long oligonucleotide called as capture strand which is complementary to mRNA of interest and
reporter strand which is a short oligonucleotide consisting fluoroophore. These both long and short oligonucleotide are hybridized and attached to gold nanoparticle for maximum quenching of fluorescence. Detection of target mRNA starts with the sequence specific binding of capture strand to its complementary target sequence in live cell. In the event that probe interrogate the target mRNA molecule and bind to the capture strand displacing the reporter strand. The reporter strand leaves the gold core and shows fluorescence as shown in figure 4.[5][6]

2.4.3. Detection and analysis
Nano flare probes are best detected by using fluorescence-based detection with single cell resolution as shown in figure 5. This consist of flow cytometer, image-based cytometers and fluorescence microscope. As the signal obtained is higher intensity platform i.e. those with laser based light sources which is better for the Nano flare detection.[5][6]

2.5. Smart Flare™ Based on Nano flare Technology
Smart Flare™ is based on the Nano Flare technology developed by Dr. Chad Mirkin.

RNA detection traditionally requires transfection, laborious sample preparation, RNA detection based on standard curves. In contrast, Smart Flare™ RNA Detection Probes enter in live cells by existing cellular endocytosis. Simply add Smart Flare™ Probes to cultures, incubate overnight and detect the next day. Over the time, the probes exit the cell, without any adverse effects.

Marketed Dosage form: Lyophilized Nanoparticle Product.

Trade mark: Smart Flare™ by Merck Millipore.[6]

2.6. Preparation of Smart Flare™ reagent: (reconstitution of lyophilized reagent)
- Add 50 µL sterile, nuclease-free water in lyophilized material in drop wise manner.
- Shake it till lyophilized material get dissolved.

2.7. Summary of basic procedure to detect cancerous cell
i. Add 60-80% population of cells to multiwall culture plates.
ii. Prepare Smart Flare™ reagent.
iii. Add reagent to cells (4uL).
iv. Incubate overnight (16 hours).[7]

2.8. Storage and precaution while handling Smart Flare™ reagent
2.8.1. Lyophilized material
- Stable for five years at 2-8 °C degrees in lyophilized form only.
- After reconstitution, store at room temperature for up to 1 year.[7]

2.8.2. After reconstitution of probe
- Probe should reconstitute with sterile nuclease free water.
- After reconstitution, stock solution should be stored at room temperature (23-27°C), protected from light.
- Product is sensitive to cold and hot temperature.
- Don’t freeze or refrigerate reconstituted material.
- Product must be handled with gloves otherwise it can be absorbed by skin and it can be harmful.[8]

2.9. Advantages
i. There is no need to use any chemical or electrical methods to force the probe into the cell.
ii. No toxicity or effect on gene expression.
iii. This approach greatly simplifies the process of identifying cancer cells.
iv. The detection of diseases at an earlier stage than current techniques.
v. This provides the potential of stopping a disease earlier, possibly with less damage to the patient.

2.10. Disadvantage
- After reconstitution of lyophilized nanoparticles stability decreases from 5 years to 1 year. [9]

2.11. Application
- Use for early detection of cancerous cell.
- Cell sorting
- Gene profiling
- Real time drug validation studies
- Gene expression [10][11]

3. CONCLUSION
The above study depicts use of novel Nano flare technology as a tool for easy detection of cancer cells in body which may be quicker method for identification of cancer and allowing to heal them at faster rate with different therapies.

4. CONFLICT OF INTEREST
There is no conflict of interest

5. ACKNOWLEDGEMENT
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6. REFERENCES


Figure 1 Structure of Nano flare

Figure 2 Working of Nano flare probe.

Figure 3 Entering Nano flare probe into the live cell.
Figure 4 Interrogate target specific mRNA

Figure 5 Detection and analysis