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Current Advances in Nanotechnology for the Next Generation of Sequencing (NGS)

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Abstract

This communication aims at discussing strategies based on developments from nanotechnology focused on the next generation of sequencing (NGS). In this regard, it should be noted that even in the advanced current situation of many techniques and methods accompanied with developments of technology, there are still existing challenges and needs focused on real samples and low concentrations of genomic materials. The approaches discussed/described adopt spectroscopical techniques and new optical setups. PCR bases are introduced to understand the role of non-covalent interactions by discussing about Nobel prizes related to genomic material detection. The review also discusses colorimetric methods, polymeric transducers, fluorescence detection methods, enhanced plasmonic techniques such as metal-enhanced fluorescence (MEF), semiconductors, and developments in metamaterials. In addition, nano-optics, challenges linked to signal transductions, and how the limitations reported in each technique could be overcome are considered in real samples. Accordingly, this study shows developments where optical active nanoplatforms generate signal detection and transduction with enhanced performances and, in many cases, enhanced signaling from single double-stranded deoxyribonucleic acid (DNA) interactions. Future perspectives on miniaturized instrumentation, chips, and devices aimed at detecting genomic material are analyzed. However, the main concept in this report derives from gained insights into nano chemistry and nano-

optics. Such concepts could be incorporated into other higher-sized substrates and experimental and optical setups.

1. Introduction

Over the last years, research into nanotechnology has shown to be of major importance in basic and applied science such as in precision nanomedicine. nano-oncology, and smart responsive nano-optical platforms for targeted detection of biomolecules at different concentration levels. These optical active nanoplatforms could also be incorporated into substrates for devices and miniaturized instrumentation. Functional nanoarchitectures could be designed with particular properties for molecular sensing. Modified metallic nanoparticles such as gold, silver, and materials such as core templates with molecular spacers with controlled dimensions could produce nano-optical platforms for chemical surface modifications.

In all cases mentioned, signal detection, transduction, and translation through space and time are required. In addition, the high sensitivity of optical platforms for single-molecule detections (SMD) and different optical setups coupled to the molecular systems under study is well known Therefore, nanotechnology is a multidisciplinary research field that responds to challenges in one of the most significant research areas, namely genomic sequencing and the next generation of sequencing (NGS) in progress. In this ongoing technology, tuned nanomaterials combined with enzymes could develop new strategies for DNA extraction, separation, detection, and quantification with microbeads. Thus, the DNA library was diluted to single-molecule concentration, denatured, and hybridized to individual beads containing sequences complementary to adapt oligonucleotides. Thus, the beads were compartmentalized into water-in-oil micro-vesicles, where clonal expansion of single DNA molecules bound to the beads occurred during emulsion PCR. After amplification, the emulsion was disrupted, and the beads containing clonally amplified template DNA were enriched.

Miniaturization of technology should be underlined to focus analysis and data recording towards individual events such as nucleotide interactions and DNA hybridization. The development of beads and nanoplatforms forming part of more complex functional machineries is applied.

It is known that sequencing addresses many challenges such as mismatching Now concentration in real samples, signal detection after complementary DNA interaction sample handling, clean-up of samples, and manipulation and multi-step procedures, all varying according to the methodology used. As could be observed, many variables should be controlled to design a new targeted methodology. This review discusses new developments from nano-optics and nanoscale control of materials with potential applications in NGS. In the first place, the basis of PCR the most widely used technology in NGS should be well understood, where nanotechnology is incorporated and, at the same time, requires new approaches based on different physical and chemical phenomena.From these perspectives, recent nano-optic developments have been discussed that center on sequencing nanotechnology based on fluorescence, synthetic non-classical light, luminescence, and enhanced phenomena by controlling high-intense electromagnetic fields from the nanoscale.

2. Current Technologies towards the Next Generation of Sequencing

For neophytes in this technique, the PCR method consists of a complex system based on enzymatic engineering that can read targeted DNA and incorporate complementary oligonucleotides by nucleophilic substitution. From very low oligonucleotide concentrations found in real samples, concentrations may be increased to levels that could be detected and quantified by a colorimetric technique. This could be regarded as the most well-known methodology used on the market; however, it is time-consuming and produces high costs linked to the use of specific biological and chemical reagents. For these reasons, the development

of modified methodologies and other derivative methods based on PCR arouses increasing interest. This technique allows the provision of an important solution to detect and quantify low genomic concentrations in real samples. This is achieved by the amplification of the genomic material involving the copy of DNA by an enzymatic strategy; hence, a resulting concentration improves the signal increase in the presence of tuned nanostructures. Many cycles could be repeated to control the desired quantity. However, the extra procedures add more time to the method. In addition, to improve time and procedures, other related PCR-based methods have also been developed, such as efficient polymerase chain reaction assisted by metal–organic frameworks. It was demonstrated that UiO-66 and ZIF-8 not only enhanced the sensitivity and efficiency of the first round of PCR but also increased the specificity and efficiency of the second round of PCR. Moreover, the modified PCR method could widen the annealing temperature range of the second round of PCR, probably due to the interaction of DNA and Taq polymerase with MOFs. A potential candidate for enhancing PCR is thus offered, yielding insights into mechanisms for improving nano-PCR and exploring a new application field for MOFs.

Accordingly, the accurate and controlled aggregation by highly specific and targeted DNA interactions could yield particles of varied sizes at the nanoscale and towards the microscale and higher dimensions. In this regard, recent high-tech developments have taken place in DNA sequencing that are closely related to NGS technologies offered on the market, such as nano-ball technology. This technology was initially developed from design of self-assemblies and nano-arrays, as in the case of the human genome sequencing using unchained base reads in self-assembling DNA nanoarrays. Regarding to the higher sized micro-structures previously mentioned, fluorescent structural DNA nanoballs have been reported for sequencing in NGS. Nanoballs are DNA self-assemblies at the nanoscale and higher scales within the microscale, with particular

properties such as nucleotide transporters and bright light sources after targeted interactions. The design considers the incorporation of intercalating fluorophore in DNA strands. It could also be used as a source of nucleotides for DNA polymerization reactions, thus amplifying local concentrations of genomic materials in real time. Highly labeled DNA nanoballs functionalized with phosphate-linked nucleotide triphosphates (dNTPs) were developed as nanoplatforms of dNTPs for DNA polymerase. The particles were prepared by strand-displacement polymerization from a self-complementary circular template. Imaged by atomic force microscopy, these functionalized particles appear as condensed, fuzzy balls with diameters between 50–150 nm. They emit a bright fluorescent signal detected in 2 msec exposures with a signal-to-noise ratio of 25 when imaged using a TIR fluorescence microscope.

In order to highlight fluorescence techniques, it should be noted that fluorescence signaling in all cases showed intrinsic high-sensitive intensity. This particular property is not shown as high from non-labeled genomic materials; for this reason, it should be added in some part of the method. This addition was by using varied fluorophores, laser dyes, and emitters with different nominations depending on the current status of the development in this research field. The fluorescence signal was thus tracked after full complementary nucleotide interactions. Both steps showed to be key phenomena to detect complementary nucleotides. In view of this, the method should rely on previous information such as known genomic probe and non-classical light wavelength to measure the targeted detection, and optimally, a signal modification should be produced after genomic material detection. These three conditions could vary according to the strategy of detection, even if fluorescence is applied as a unique detection technique. Challenges posed in these three steps are connected with real-sample cleaning and experimental procedures such as chemical conjugation, labeling, and interference. Potential molecular optical active biomolecules could quench emissions and

hinder oligonucleotide detections. Thus, the application of fluorescence varies by developing labeling or biolabeling with bioconjugation techniques. Associated methods such as direct fluorescence emissions, FRET, FISH, incorporation of more complex enzymatic bio machineries, as well as the development of accurate targeted aggregation have proven to be new ways to overcome difficulties in genotyping.

To conclude this section, fluorescence techniques have been used to accomplish sequencing from the molecular level to higher-sized nanochemistry control and participate in nucleotide chemistry and DNA interaction. In this particular research field, it is very important to examine strategies already developed and transfer high-impact research in real applications to provide innovative ways to address the current challenges linked to low DNA concentrations in real samples for detection and quantification.

3. Enhanced Techniques and Methods for Sequencing and Genotyping

With the aim of developing new strategies for DNA detection to enhance current techniques and methods, approaches from aptamer-based point-of-care diagnostic platforms are in progress. In these synthetic systems, DNA strands of varied lengths are incorporated in different bio-molecular machines where signaling is recorded after targeted interactions based on non-covalent bonding. In view of this, a broad range of well-established and novel diagnostic platforms is being considered for use in commercial point-of care (POC) diagnostics employing aptamers instead of antibodies as molecular recognition elements where light turns on the detection. In this case, an enhancement strategy is required to highlight photodetection. In this context, it could be mentioned briefly that from other developments and strategies, ideas and concepts could be taken and be transferred to new designs and developments focused on

genotyping. Therefore, the grafting of surfaces and substrate modification to generate microarrays on, for instance, glass slide coating, has been largely used and demonstrated. Then, this capability allowed proposing other approaches in addition to new portable technology such as selective, aptamer-based, and ultrasensitive nanogold colorimetric smartphone readouts for detection of heavy metal ions. Additional functions were also incorporated as well, such as targeted bioimaging and photodynamic therapy nanoplatform using an aptamer-guided G-Quadruplex DNACarrier and near-infrared light by a selective system that delivers a photosensitizer to targeted cells and upon irradiation. These developments showed improved properties not found in other developments reported. This improvement results from the incorporation of targeted components in a complex functional structure. The concept could be transferred to DNA biosensors incorporating different physical and chemical strategies and serving as a synergic methodology, enhanced pathway, and chirped laser mechanism.

Note the tuning of fluorescence to develop new amplified signals such as biolasers and living lasers. The concept is relatively easy to understand; however, generating phenomena related to broader applications proves more complex. Biolasers are generated from tune emissions of natural or synthetic dye emissions by controlling their media in biostructures such as protein complexes, where the dye is incorporated in a protective cage of the excited state. Thus, amplified signaling could produce increased and stable emission in biological media such as green fluorescent protein. This is a protein that exhibits bright green fluorescence when exposed to ultraviolet blue light. This cage, like a stable structure, showed targeted emission wavelengths modifying their biostructure and dyes, often referred to as variants of green proteins. Due to the high sensitivity against medium modifications, these fluorescent proteins were used as reporters of gene expression, and contaminants were used as heavy metal ions. They were also shown to detect variation

of different cell stress levels of zebrafish. This small-animal model injected with the green protein was no less than twenty times more susceptible to recognizing cellular stress as compared to that not injected with this protein.

It is important to mention the implication of new properties that could lead to meta-materials and non-classical properties where photons are modified after matter interactions. In this way, nanotechnology provides many approaches based on the combination of coupled phenomena with enhanced light generation and potential bio-applications. Hybrid silica multi-colored enhanced fluorescent nanoparticles were recently developed from FRET and incorporated into two laser dyes in a confined nanoscale volume. It was thus possible to tune light emissions and intensities according to the laser excitation used. This nano-emitter was applied to non-classical light delivery in unicellular microorganisms such as cyanobacteria. This led to the generation of synthetic non-classical luminescence by enhanced silica nanophotonics based on nano-bio-FRET. This effect was controlled by the laser excitation applied and energy-transfer pathway activated with optional higher and lower quantum yields according to the natural protein photo-system coupled to the biostructure

DNA detection for early diagnosis in real tissues is also worth mentioning. A DNA bioassay was based on a template-directed and labeled primer detected by FRET. This methodology was identified as template-directed dye-terminator incorporation (TDI) assay. Thus, it achieved the detection of mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, the human leukocyte antigen H (HLA-H) gene, and the receptor tyrosine kinase (RET) proto-oncogene associated with cystic fibrosis, hemochromatosis, and multiple endocrine neoplasia type 2, respectively. The method consisted of steps such as PCR amplification, enzymatic degradation in the excess of primers, and

deoxyribonucleoside triphosphates before performing primer extension reaction. However, all these standardized steps were performed in the same tube, and the fluorescence changes were monitored in real time, providing insights into future biosensors and bioassay developments.

To understand and improve the phenomena occurring in these particular energy-transfer processes in complex matrixes with optical active biomolecules in their close surroundings, many developments have been reported in the control of the nanoscale by genomic nanomaterial formation. A case in point is the dual-channel single-molecule FRET-based dynamic DNA-detection system to establish distance parameters in RNA nanoparticles. As known, FRET is highly dependent on and sensitive to the distance of the donor/acceptor emitter species. In search of genomic material imaging, systems with nanometer-scale resolution for RNA detection were studied. Hence, Phi29 dimeric pRNAs can serve as building blocks in assembly of the hexameric ring of the nanomotors, as modules of RNA nanoparticles, and as vehicles for specific therapeutic delivery to cancer or virally infected cells. In this particular complex biological system, they was calculated and used as distance parameters to optimize a known reported 3D model of the pRNA dimer. Distances between nucleotides in pRNA dimers were therefore found to be different from those of the dimers bound to procapsid. This difference results from a conformational change of the pRNA dimer upon binding the procapsid, which accounts for how biological media could differently affect energy transfer and consequent aptamer detection in further studies of genotyping applications. Fluorescent RNA aptamers could be useful as biolabelers for detecting and tracking RNA molecules into cells. One study shows a genetically encodable single-stranded RNA origami scaffold using fluorescent RNA aptamers. To record a FRET-based detection signal, fluorescent aptamers were placed in close proximity to RNA scaffolds, and a new fluorophore was synthesized to increase spectral overlap. The nanoarchitecture

obtained acted as an RNA device causing conformational changes in the presence of all the components, by means of which an apta-FRET signal was recorded. This phenomenon was expressed in controlled genetically engineered *E. coli*, demonstrating that the apta-FRET system was genetically encodable and that the RNA nanostructures fold correctly in bacteria.

Moreover, DNA origami based Förster resonance energy transfer nanoarrays and their application as ratiometric sensors have also been reported. In this approach, DNA acted as the main building block of an optimal targeted nanoarchitecture formation only in the presence of the full complementary DNA strand and the tunable coupled fluorescent dye incorporated. The dyes were arranged at accurate distances, where they efficiently interacted by energy transfer. In this study, the high-bright fluorescent nano-origami was applied as a pH sensor. The brightness and sensitivity of a ratiometric sensor were improved simply by arranging the dyes into a well-defined array.

In this regard, the generation of smart responsive surfaces from nanoarrays to larger modified arrays in the nanoscale control is highly required in many current technological approaches and technology centered on DNA detection and genotyping. Numerous strategies to generate signal modifications from the molecular level can be made possible by combining proper chemical surface modification by wet chemistry methods, nano-patterning, and coupling of appropriate optical setups. Therefore, targeted aptamer detection and quantification could be possible to achieve. Thus, the manipulation of low DNA concentration is the greatest challenge to overcome. The strategies to solve this issue and related ones are of high interest and impact in this field. Research into single-molecule detection (SMD) with applications in DNA targeting has been reported. A single-step FRET-based detection of femtomole DNA was recently developed. This development was based on recyclable platforms of the toehold-

mediated strand displacement (TMSD) process, leading to a distinct change in FRET efficiency upon target binding, which allowed a detection of a low femtomole DNA concentration without needing/requiring target amplification. The method involved manipulation of small sample sizes (fewer than three orders of magnitude compared to the typical sample size of bulk fluorescence). Furthermore, these single-molecule sensors exhibited a dynamic range of about two orders of magnitude. Thus, an evaluation of high sensitivity was carried out at the level of nucleic acid detection and identification of the single-nucleotide polymorphism (SNP), which is crucial in diagnosis of genetic diseases.

Finally, and opening the discussion of new strategies and optical setups, developments have been made by miniaturizing larger modified surfaces in microarrays, reduced-size devices, and chips. Studies have shown ultrasensitive DNA detection in microarrays by fluorescence labeling without material amplification and detection by fluorescence imaging with a single dye sensitivity With this approach, single dye molecules can be reliably detected with an average signal-to-background-noise ratio of ~42, and this result was achieved by a simple chemical modification of aldehyde surfaces. Then, fluorescence-labeled complementary oligonucleotides were hybridized at various concentrations, enabling the control of femto-molar oligonucleotide concentrations. Thus, 10 fM concentration signals of individual, specifically hybridized oligonucleotide molecules were resolved. In this way, it was shown how strategy and optical setups could be managed to provide a conceptual basis of bioassays for expression profiling of low amounts of sample material without signal amplification. Hence, there is a huge potential of DNA nanotechnology for non-classical light generation, tuning, light harvesting, signal enhancements, and biosensing developments focused on DNA detection.

4. Advances and Perspectives from Nanotechnology towards NGS

The discussion of perspectives on NGS from the nanomaterial viewpoint is closely related to current nanotechnology developments. From fundamental research with new concepts and designs, proofs of concepts can be discussed and evaluated as derived from real technology. In this perspective, and knowing that NGS is focused on the determination from single nucleotides to the accurate order of variable nucleotide composition within longer DNA or RNA chains. we should highlighted its potential impact and capability to provide insights within other research fields.

Therefore, there are still challenges to address in relation to the main variables described as well as new ones related to further capabilities using new detection strategies and systems. In this section, we show some representative high-impact nanotechnology developments in progress, encouraging innovation in DNA-detection and -genotyping technology.

As known, sequencing requires facing many challenges ranging from low concentrations in real samples and isolation to amplifying genomic material. The amplification procedure increases the quantity of the genomic material by copying targeted sequences. The generation of DNA-strand libraries allows continuing the design of detection strategies. Then, even if many genomic libraries are available, variable epigenetic detection could add extra difficulties. Thus, real samples by intrinsic and natural expression could affect determinations as well, and it should be contemplated in the design of the methodology. In this context, DNA mismatching should be solved. Probably, the most important variable to transduce and generate specific detection signals from single-nucleotide interactions is based on non-covalent forces. Considering natural and non-synthetic nucleotides, DNA detections from short aptamers to longer genomic chains are generally based on complementary double-stranded DNA.

Moreover, other important variables such as manipulations, clean-up, sample handling, and multi-step procedures comprising each methodological step could affect efficiency and yield, and they should be considered as well. By this manner, a multivariable system could be contemplated that could reveal additional factors on the road ahead in genetics and genomics research.

To find new solutions to the needs and challenges described, a multidisciplinary research field should be opened up. However, the control of the nanoscale and nanotechnology production could lead to new approaches and proofs of concepts with potential transfer to NGS technology.

Enzymatic machineries can be used to amplify genomic material such as the well-known polymerase chain reaction (PCR). For example, a rapid and efficient DNA isolation method was developed for qPCR-based detection of pathogenic and spoilage bacteria in milk. For neophytes, qPCR is a modified PCR-based technology that allows quantifying real-time single-oligonucleotide reading using fluorescent reporter molecules in targeted quantifications.

Robust technology has been developed based on natural complex enzymatic machineries such as PCR. Recently, a gene-based precision medicine technology known as clustered regularly interspaced short palindromic repeats (CRISPR) was created. This biotechnology was developed to repair genomic material by incorporating new oligonucleotide sequences. This is based on a complex enzymatic system that acts as an enzymatic scissor in targeted DNA sequences coupled to the replacement of genomic material. Recently, two researchers working at Max Planck Unit for the Science of Pathogens, Germany, and at University of California, USA, were awarded the Nobel Prize in Chemistry 2020 for “the development of a method for genome editing”. Therefore, CRISPR/Cas9 is still being studied to modify the DNA of animals,

plants, and micro-organisms with extremely high precision. This technique, which is able to control fragmentation and re-incorporation of new genomic material, is particularly interesting when transferred to new strategies for DNA detection. Thus, CRISPR could generate different DNA-detection systems by adding fluorescence labelers.

From the chemical modification of nucleotides and DNA, RNA strands have provided further perspectives by integrating genomics with precision medicine. There are many relevant research studies; however, the combination of a specific nucleotide interactions accompanied by pharmacophores linking and bio conjugations for targeted drug delivery perspectives should be briefly highlighted. Moreover, by this manner, non-covalent interactions are related in the targeted function.

By exploiting the concept of non-classical light from the molecular level by fluorescence labeling such as DNA-intercalating agents or by covalent bond modifications, the research work for new imaging-based developments should be continued. Recently, research has shown an integrated imaging and computational strategy to model gene folding with nucleosome resolution. It was thus possible to identify a specific distribution of nucleosomes within specific genes in super resolution through the simultaneous visualization of DNA and histones. This method advanced information on chromatin accessibility for regulatory factors such as RNA polymerase II. Intercellular variability, transcriptional-dependent gene conformation, and folding of housekeeping and pluripotency-related genes were studied in human pluripotent and differentiated cells, gathering accurate data.

To develop further resolution to nano-biostructures, the molecular level, from the bottom-up design that could provide targeted bio molecular detection from proteins to amino acids and small molecules, should be evaluated. Similarly, the resolution of

single nucleotides could prove a major development; yet, this level of resolution is only achieved by a highly smart responsive strategy. In this context, biophotonic strategies for single-molecule detection (SMD) level should be considered. Therefore, there is a growing body of literature on DNA detection and amplification based on different optical approaches and controlled DNA grafting of surfaces. For single-molecule sequencing, we may refer to the sequencing and tracking of individual nucleotides based on templated DNA exposed to a solution containing DNA polymerase and a fluorescent nucleotide. If a nucleotide were incorporated, it would be achieved by a complementary strand of the template. Thus, the fluorescence would be read using a total internal reflection fluorescence (TIRF)-based hagioscope, recording the positions where the DNA strand had incorporated a fluorescent nucleotide from the solution. It should also be noted that real-time DNA sequencing from single polymerase molecules enabled tracking of single-nucleotide incorporation in real time by fluorescence imaging. The level of developments achieved in the single-molecule dynamic detection of chemical reactions based on an electrochemical device is also worth noting.

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