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DNA Detection *via* Nanoparticles

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The detection of DNA is a highly promising field of research. The ability to detect and classify DNA according to the organism it originated from has numerous worthwhile applications in medicine. The importance of this research can be seen in the following examples, firstly: the detection of harmful bacteria on surfaces, the detection and elimination of harmful bacteria leads to a sterile and safe hospital environment. The second is the detection and classification of harmful bacteria present within the human body. Early detection can lead to the treatment and removal of disease before symptoms appear. Nanoparticle-enabled detection of DNA is able to detect picomolar concentrations of target DNA (Spain 2012). This paper will focus on current techniques of DNA detection in nanoscience.

Colorimetry or Polymerase Chain Reaction?

Colorimetry is a currently viable method of detecting DNA with nanoparticles. Colorimetry is characterized by a change in colour of a solution as reactions proceed to completion. In general, colorimetry is a less sophisticated and less sensitive method of detection compared to fluorescence-based polymerase chain reactions (PCR). PCR is a very sensitive method of DNA detection, capable of amplifying single DNA molecules. DNA is detected through PCR through electrophoresis and ethidium bromide staining. One issue with PCR is that it is difficult to selectively detect DNA, it is prone to false detections of target DNA (Dennis 1998). The discrepancy in sensitivity between colorimetry and PCR can be decreased with the aid of nanoparticles, while maintaining the simplicity and low cost of colorimetry based detection methods (Shen 2012).

Gold Nanoparticle Enabled DNA Detection

Gold nanoparticle DNA assays are less sensitive than real time PCR, thus a method to increase their sensitivity is needed; the in situ amplification of gold nanoparticle aggregations along with target DNA is required (Shen 2012). Gold nanoparticles are citrate-capped and coated with capture probes. These gold nanoparticle assemblies are then ligated in a mixture containing ampligase, the nanoparticles themselves, partial probes and the target DNA. This mixture is sent through a thermal cycle, which involves hybridization and ligation at 50°C and denaturation at 90°C. In the hybridization stage the target DNA and probes duplex with the gold nanoparticles, while the ampligase in the mixture concurrently ligates two gold nanoparticles. At the denaturation stage of the cycle, the mixture is heated to 90°C. At this temperature the formed duplexes are denatured, which releases the target DNA, the partial probes and the ligated gold nanoparticles (Shen 2012). With each thermal cycle the number of ligated gold nanoparticles increases by a factor of two. After sufficient thermal cycles, the colour of the solution changes from a dark red to purple at relatively low concentrations, and to gray at relatively high concentrations (Shen 2012). When no target DNA is present, or is non-complementary to the capture probes on the gold nanoparticles, no change in colour of

solution is observed. This signifies that this method of DNA detection is effective. At 90°C, this method of DNA detection is able to clearly detect target DNA whilst mixed with genomic DNA which is 100,000-fold larger in size. This highlights the selectivity of this method of DNA detection and broadens its potential uses in industry and medicine.

Platinum Nanoparticle Enabled DNA Detection

Spain and colleagues' work with platinum nanoparticles and DNA detection had the goal of being able to detect DNA without requiring amplification (Spain 2012). DNA amplification is costly and time consuming; a direct detection of DNA, in this case via the detection of electrochemical changes, is more efficient and avoids the need for molecular amplification. It is well known that platinum nanoparticles catalyze the reduction of hydrogen peroxide (You 2012; Katsounaaros 2012). Single stranded probe DNA is bound to the top side of mushroom-shaped platinum nanoparticles, which are held in place in self-assembled monolayers of dodecanethiol. The platinum nanoparticles are desorbed and released into a solution, containing hydrogen peroxide, by a jump in current. These platinum nanoparticles are capable of binding with target DNA on the top side, whilst still being electrocatalytic. An additional electrode, which has a complementary sequence of DNA to the target DNA, and an additional area available to bind to the probe DNA on the platinum nanoparticles is added to the solution. In this manner, binding of the platinum nanoparticles to the target DNA is quantified by a current associated with the reduction of hydrogen peroxide. Target DNA concentrations ranging from 10pM to 1μM are able to be detected through this method (Spain 2012).

Using this method it is important to regionally functionalize the platinum nanoparticles. When the nanoparticles were homogeneously functionalized with the single stranded probe DNA, the current detected by the electrode was significantly lessened compared to the isolated top-sided probe DNA. The sensitivity of the homogeneously functionalized platinum nanoparticles is roughly 5 times less than the sensitivity of the regioselectivity functionalized particles. This result shows that while the homogeneously functionalized particles are still acceptable as a DNA detection method, the regionally functionalized particles deliver a much more sensitive result, and are thus worth the extra preparation (Spain 2012).

The platinum nanoparticle enabled DNA detection method is also highly selective. With one base-pair mismatch on the DNA sequence, the change in current dropped by a factor of four. Furthermore, a mismatch of three different base-pairs resulted in no measureable current (Spain 2012). The selectivity of this method of DNA detection is important. It allows one to differentiate between different bacterium, especially between those that are harmful, or have harmful relatives and those that are benign. In Spain and colleagues' work they were able to differentiate between *Staphylococcus epidermis* and *Staphylococcus aureus*, two bacteria which have three nucleotide base mismatches (Spain 2012).

Conclusion

The need to develop reliable, safe, and sensitive DNA detection methods is massive. The potential effects of the development of these methods are equally as important – their applications will undoubtedly save lives. Colorimetry based DNA detection has progressed substantially now that gold nanoparticles have been investigated. Previously colorimetry was limited in that it had low sensitivity, it was capable of detection of DNA to micromolar

levels; with the onset of nanoparticle research in this field, the sensitivity of colorimetry has increased to detection levels of nanomolar concentrations (Shen 2012). Further advancement of this colorimetric detection method would be to find a way to directly detect DNA without the need to amplify it. This would reduce associated costs, the time involved and make the method easier to perform (Shen 2012). The research performed by Spain *et al.* provides a simple, cost-effective, sensitive and highly selective method of DNA detection. Advancement of this form of DNA detection involves the development of a more efficient method of desorption of the functionalized platinum nanoparticles; in doing so, the sensitivity of this method of DNA detection will increase (Spain 2012).

The ability to detect and classify harmful bacterial strains before their symptoms appear in a subject is a worthwhile area of research to pursue. It would allow the delivery of strain-specific drugs, never allowing the effects of the bacteria to surface. The potential of these nanoparticle-enabled DNA detection methods in anti-bacterial and anti-viral applications are apparent. As further research is conducted the results of these methods will hopefully be realized in hospital environments, and anywhere else that requires highly selective and sensitive detection of DNA.

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