**Engineered biopolyester beads as a tool for specific detection of global DNA methylation**

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DNA methylation is a significant epigenetic mechanism that plays an important role in various biological process, such as DNA repair, gene transcription, and embryogenesis 1. Recent studies show that abnormalities in DNA methylation patterns presents a signature for disease diagnosis, prognosis of outcome and therapeutic interventions. However, analysis of methylation patterns in genome is significantly challenging due to the complex nature of the samples and the relatively low abundance of the targeted DNA. In recent years, many nanotechnology-based approaches have been developed for DNA methylation detection using metallic nanoparticles, carbon or metallic nanotubes, magnetic particles, and functionalised conductive polymers. However, most of these technologies are suitable for analysing DNA at a single allele methylation.

We have recently synthesised a new class of tunable, superparamagnetic, biopolyester beads which could be used as (i) dispersible capture agents 2, and (ii) as surface modifiers for detecting circulating biomarkers in complex biological fluids. Polyhydroxyalkanoate (PHAs) composed of hydroxy fatty acids, can be synthesised by various bacteria and archaea, and act as their energy and carbon storage. These are deposited into the cytoplasm as insoluble spherical inclusions. PHAs offer several advantages including their biodegradable and thermoplastic properties, renewable, elastomeric, modifiable and biocompatible. Herein, we report a novel, *in vivo* self-assembling and inexpensive platform method for the analysis of DNA methylation based on PHA beads. The PHA beads displaying protein binding domains are modified with horse-radish peroxidase (HRP)-tagged methylation-specific (5-mC) antibody that can specifically bind with the methylation’s sites within the single stranded DNA sequences. The captured ssDNA are then magnetically purified, isolated, and directly adsorbed on the gold surface via gold-DNA affinity interaction.3-5 PHA beads can also be directly adsorbed on the gold surface via self-assemble monolayers followed by direct immobilisation of target global DNA onto the bead surface. HRP-tagged 5-mC antibody was then immobilized with the PHA-bound global DNA. The enzyme-catalysed colorimetric reaction in the presence of 3,3’,5,5’-tetramethylbenzidine (TMB)/H2O2 system was employed to generate colour change for quick naked-eye observation, as well to achieve semi-quantitative analysis by UV-visible spectroscopy. As the catalytic reaction produce electroactive coloured complex, it was also used to attain global DNA methylation level by chronoamperometric measurements. The assay could successfully differentiate changes in DNA methylation as low as 5%. Furthermore, this method has also been used to detect methylation levels in fresh tissue samples from patients with ovarian cancer. We believe that this inexpensive, rapid, and sensitive assay could potentially be used as a low-cost alternative for DNA methylation analysis in clinical applications.

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