Filamentous phage-derived nano-rods for applications in diagnostics and vaccines

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Dedicated to my parents

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"In the name of Allah, the Most Gracious, the Most Merciful"

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Abstract

Filamentous bacteriophage, as their name indicates are filament-like bacterial viruses. The F-pilus-specific filamentous phage of *Escherichia coli*, Ff (f1, M13 and fd) are resistant to heat, pH extremes and detergents. Their structural properties and amenability to engineering using recombinant DNA technology have enabled their extensive use in modern biotechnology. For example, Ff can be functionalized by displaying up to five different proteins and peptides on their surface. Ff phage have been successfully employed in diagnostic devices. Moreover, direct use as antigen-carriers is also a subject of interest in vaccine development. However, use of Ff-phage vaccines and in the at-home diagnostic devices is controversial, mainly because of their ability to replicate in gut *E. coli*, and possibility of mobilization and horizontal gene transfer of antibiotic resistance or virulence factor-encoding genes transfer among the gut and environmental bacteria. Moreover, the large length-to-diameter ratio of the virion (1000 nm x 6 nm) impairs diffusion of filamentous phage through complex matrices and could restrict use of filamentous phage in lateral flow diagnostic devices.

To overcome both of these problems we have constructed much shorter, rod-like functionalized particles (50 nm x 6 nm), named “Ff-nano”, which do not carry any genes. The properties of these short particles were investigated, showing that they have superior resistance to heating in the presence of ionic detergent sodium dodecyl sulphate (SDS) in comparison to the full-length phage of the same virion composition. The Ff-nano particles displaying a bacterial Fibronectin-Binding (FnB) protein as fusion to virion protein pIII, localized in five copies at one of the two ends of the virion, were produced and purified. These functionalized nanorods were tested in two applications: as detector particles in a dip-stick-type lateral flow device and as antigen carrier in a vaccine trial. The FnB-displaying nanorods were able to quantitatively detect fibronectin in solution. In the vaccine trial, the Ff-nano particles elicited a weak response to the FnB displayed at a low-copy-number at the nanorod end. In contrast, the response to the major protein pVIII was strong, indicating that the multi-copy display of antigenic peptides along the rod, as fusion to the major coat protein pVIII, is required for using the Ff-nano effectively as vaccine carriers.

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