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Adsorption of T4 bacteriophages on planar indium tin oxide surface via controlled surface tailoring

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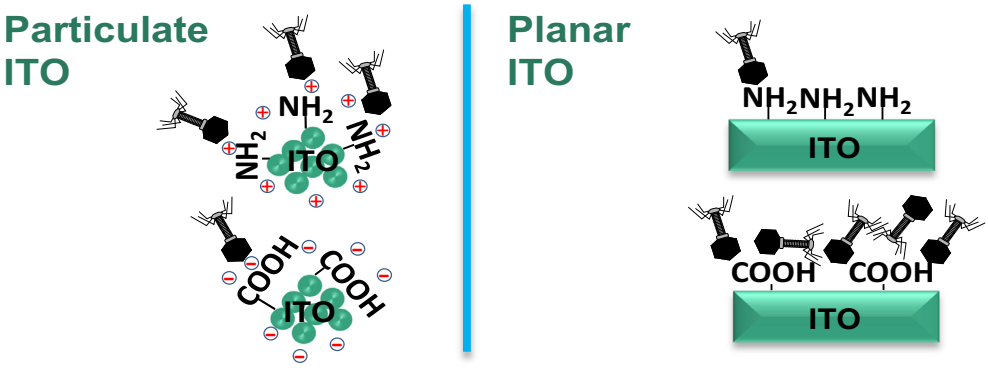
GRAPHICAL ABSTRACT



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ABSTRACT

The work investigates the influence of surface physicochemical properties of planar indium tin oxide (ITO) as a model substrate on T4 bacteriophage adsorption. A comparative T4 bacteriophage adsorption study shows a significant difference in bacteriophage adsorption observed on chemically modified planar ITO when compared to similarly modified particulate ITO, which infers that trends observed in virus-particle interaction studies are not necessarily transferrable to predict virus-planar surface adsorption behaviour. We also found that ITO surfaces modified with methyl groups, (resulting in increased surface roughness and hydrophobicity) remained capable of adsorbing T4 bacteriophage. The adsorption of T4 onto bare, amine and carboxylic functionalised planar ITO suggests the presence of a unique binding behaviour involving specific functional groups on planar ITO surface beyond the non-specific electrostatic interactions that dominate phage to particle interactions. The paper demonstrates the significance of physicochemical properties of surfaces on bacteriophage-surface interactions.

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1. Introduction

Bacteriophages are viruses capable of identifying and infecting specific species or strains of bacteria [1]. Applications of bacteriophage, which exploit their high degree of specificity [2], and their ability to infect [2,3] and identify viable from non-viable bacteria [4,5], have received considerable interest in recent years. Potential applications have included their use in biosensors [6–8], phage-based biosorbents [9], antibacterial surfaces [3] and as a model virus in characterisation of membrane filtration systems [10]. With encouraging advances in the use of bacteriophage, controlling their interactions with material surfaces is a crucial design component. The fabrication of such surfaces necessitates a fundamental understanding of virus-surface interactions; in particular, those that govern the adsorption of virus onto the surface in a controlled manner.

A useful strategy to better understand the influence of physicochemical properties of adsorbing surfaces towards virus adsorption is through a comparative study that employs different surfaces with defined physicochemical properties. Previous studies have turned to modifying the surface chemistry of particles. It is found that virus-particle interaction is largely influenced by net electrostatic interactions between two oppositely charged components [9,11–13]. However, with the expansion of bacteriophage applications beyond the use of particulate based substrates, such as biosensors and antibacterial surfaces, an understanding of the extent to which substrate physical configuration contributes to the virus-surface interactions is yet to be established. Indeed, a number of studies of virus adsorption to surfaces have been performed that cover a variety of viruses and surface chemical properties [14–16], many of which however, are often contradictory, perhaps because of different surfaces being studied and different methods of studying these interaction being employed. For instance, Moghimian and colleagues reported the adsorption of M13 filamentous bacteriophage on to a planar carbon grid was due to high surface hydrophobicity and isoelectric point (IEP) [14]. In contrast, high surface hydrophobicity was found to have little influence to the adsorption of the same bacteriophage to a hydrophobic gold coated surface [15]. Low IEP of SiO_2 surface was found to induce poor M13 filamentous bacteriophage adsorption [14] while adsorption of dengue virus to low IEP SiO_2 wafer was found to be high [16]. The complex interactions between virus and the different physicochemical properties of surfaces give rise to challenges in deciphering the mechanism and factors influencing the virus adsorption. However, such an understanding is needed as the impact of surfaces on virus adsorption behaviour has implications for many intended applications, such as biosensors and antimicrobial surfaces.

In this work, we aim to provide a comparative study that investigates the significance of surface physicochemical properties via controlled surface modification of a planar indium tin oxide (ITO) surface as model substrate, on the adsorption behaviour of T4 bacteriophages. The study employed surface functionalisation via organosilane grafting which allows proper presentation of different chemical moieties, namely amine ($-NH_2$), carboxyl ($-COOH$) and methyl ($-CH_3$) groups, as well as the variations in their surface charge and hydrophobicity. Herein, we report unique T4 adsorption behaviour to the differently functionalised planar ITO surface beyond simple net electrostatic interaction, which has been widely documented in previous studies of virus-particle interactions [9,12,13]. Using a particulate form of ITO as a comparison, we observe for the first time the significant relevance of the physical configuration of the adsorbing surface (planar vs particulate) in dictating the T4–ITO interactions. Data presented strongly suggests that virus-particle interactions are not directly transferrable to predict virus-planar surface interactions.

2. Materials and methods

2.1. Reagents and materials

Planar indium tin oxide (ITO) coated cover slips (18 × 18 mm, 15–30 ohm) were obtained from SPI Supplies. 3-aminopropyltriethoxysilane (APTES), octadecyltrimethoxysilane (ODTMS), bovine serum albumin (BSA), ITO nanopowder (referred as particulate ITO) and sodium citrate tribasic dihydrate were supplied by Sigma–Aldrich. Analytical grade methanol, ethanol, glacial acetic acid, *N*,*N*-dimethylformamide (DMF), sodium chloride, sodium hydroxide pellets, dichloromethane, Tris–HCl, glycerol and D-glucose were purchased from Ajax Chemicals. Phosphate buffer saline tablets (8 g/L NaCl, 0.2 g/L KCl, 1.15 g/L Na_2HPO_4 and 0.2 g/L KH_2PO_4, pH 7.2–7.4, Ionic strength 298 mmol/L), beef extract, tryptone, yeast extract and agar bacteriological were obtained from Oxoid. Bacteriophage T4 (ATCC 11303-B4) and its host *Escherichia coli* ATCC 11303 were procured from the ATCC.

Luria Bertani (LB) broth was prepared by dissolving 10 g/L tryptone, 5 g/L yeast extract and 10 g/L sodium chloride in Milli-Q water. Tryptone agar was made by adding 10 g/L tryptone, 8 g/L sodium chloride, 2 g/L sodium citrate tribasic dihydrate, 3 g/L D-glucose and 10 g/L agar bacteriological) in Milli-Q water. Media were sterilised by autoclaving.

2.2. Preparation and modification of planar and particulate ITO

All ITO substrates were cleaned by immersing the ITO substrates in dichloromethane, followed by methanol for 10 min each and in 0.5 M K_2CO_3 in Milli-Q water/MeOH (1:3, v/v) for 30 min under constant sonication. The ITO substrates were rinsed thoroughly with a copious amount of Milli-Q water and dried at 110 °C.

The amine terminated ITO was prepared by immersing the cleaned planar ITO surface or 0.3 g cleaned particulate ITO into a solution containing 5 mL anhydrous methanol, 0.25 mL water, 0.5 mL glacial acetic acid and 30 mL glycerol. The solution mixture was sonicated using an ultrasonic probe (Misonix) for 1 min in a three-necked round bottom flask. A mixture of 0.5 mL APTES in 5 mL of anhydrous methanol was titrated into the flask and the solution was heated to 120 °C for 15 h under a N_2 environment. After modification, the surface was subjected to repeated washing in Milli-Q water followed by ethanol to remove any weakly bound molecules and dried under vacuum. The bare ITO was prepared by following the protocol above and replacing the 0.5 mL APTES with 0.5 mL anhydrous methanol. The bare ITO was used as a control sample.

To introduce the carboxylic functional group, amine terminated planar ITO surface or 0.08 g of particulate ITO–NH_2 was added to a succinic anhydride (10%) in anhydrous DMF. The mixture was stirred for 3 h under a N_2 environment. The substrate was washed repeatedly in DMF followed by Milli-Q water and dried under vacuum.

ITO–CH_3 was prepared by immersing pre-cleaned planar ITO surface or 0.08 g of cleaned particulate ITO in 0.5% (v/v) *n*-butylamine in anhydrous methanol. 5% (v/v) ODTMS was added slowly into the solution. The sample was sonicated for 60 min at 20% amplitude. It was then left to incubate for 30 min, before being washed three times in methanol and acetone. The sample was dried overnight under vacuum.

2.3. Phage immobilisation

All immobilisations were performed in 10 mM phosphate buffer that had been adjusted to pH 6. The T4 bacteriophage titre was