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Supramolecular scaffolds on glass slides as sugar based rewritable sensors for bacteria†

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We describe here the sugar functionalized β -cyclodextrin–ferrocene glass slides as fully reversible bacterial biosensors under the influence of external adamantane carboxylic acid. The prototype D-mannose – *E. coli* ORN 178 and L-fucose – *P. aeruginosa* interactions serve as a model to illustrate the new approach.

Food- and water-borne diseases cause over 3 million deaths every year, of which, according to WHO publication, about 90% are children younger than 5 years from poor families and communities in developing countries.¹ The major reasons for the rapid increase in global pathogen infections are strain mutations and emergence of antibiotic resistance.² However, appropriate medical treatment based on early diagnosis can decrease fatalities. Currently, detection of pathogenesis is done using DNA based PCR assay and protein-based ELISA³ techniques but these methods require trained personnel and are also influenced by the environment. A different approach for detection is based on carbohydrates that are used as biomarkers for pathogens and is based on the highly selective recognition of bacterial lectins through specific oligo-saccharide epitopes.⁴ Carbohydrate–protein interactions are weak but multivalent interactions amplify recognition.⁵ We and others have harnessed these interactions as templates for recognition of bacteria.⁶

In this communication we describe a technique to prepare a glass substrate covered with sugar dendrimers which can be used as a platform for detection of bacteria. Our approach utilizes the special high tendency of β -cyclodextrin (β -CD) to form host–guest complexes with small molecules, especially with adamantane and ferrocene molecules⁷ and the ability to

attach a multitude of sugar molecules in close proximity by attaching them to its skeleton. We have synthesized a β -CD scaffold which contains seven sugar molecules with increased carbohydrate mediated interactions. This new sugar-modified β -CD derivative was used for developing a sensor on glass substrates. The attachment of this compound to the glass surface was achieved by formation of host–guest β -CD–ferrocene complexes. The specific advantage of ferrocene over the adamantane linker is the difference in the strength of the host–guest complexation,⁷ which yields a facile method to regenerate the surface to develop a protocol for removal of the bacteria and sugar after binding so that the same slide can be used continuously for similar or different pathogen–carbohydrate interactions.

We used mannose and fucose as sugars and the specific bacterial strains mannose-FimH of *E. coli* ORN 178 and fucose-PIM-2 of *P. aeruginosa* to establish the selectivity, sensitivity and reversibility of the detection platform.⁸

Mannose-modified β -cyclodextrin (C-2) was synthesized as described previously.⁹ C-3 was synthesized from peracetylated fucose 2 which was glycosylated with bromoethanol in the presence of NIS and TFOH, converted to thiocyanate, followed by Zn/AcOH reduction to yield 3. This derivative was reacted with peracetylated hepta iodo- β -CD 4 in the presence of Cs_2CO_3 yielding 5 that was deacetylated in the presence of sodium methoxide to yield C-3 (Scheme 1).

Ferrocene derivative L-1 was prepared by coupling mono-boc protected ethylene diamine and ferrocene monocarboxylic acid, followed by deprotection using TFA in DCM (Scheme 1).

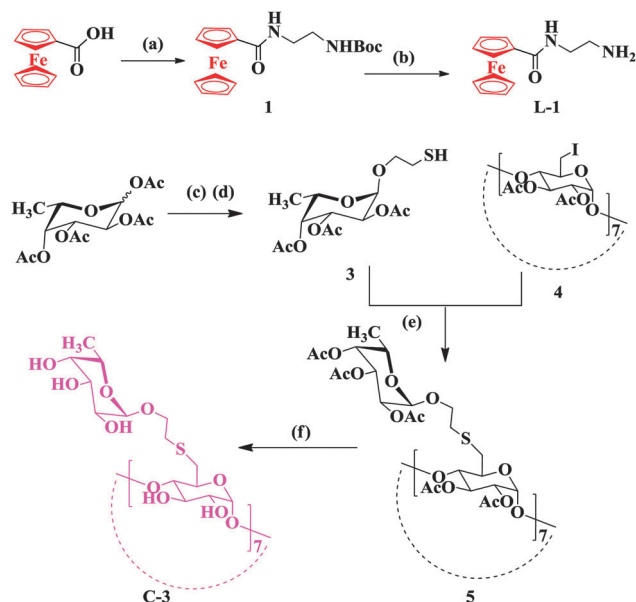
Robust ferrocene-based monolayers were formed by assembling ferrocene derivative L-1 on silylepoxide-coated glass or silicon slides. This was done by washing glass slides (approx. 1×1 cm in size) with piranha solution followed by dipping them immediately into a solution of 3-glycidyloxypropyltrimethoxysilane (GOPTMS) in toluene. The substrates were heated at 85 °C for 52 h in a pressure tube, rinsed with toluene to remove excess GOPTMS and dipped in a solution of L-1 (0.02 mM) in ethanol for 24 h. Finally the substrates were rinsed with ethanol to remove the excess ferrocene derivative and to remove residual epoxide groups (Fig. 1). The process yielded

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Scheme 1 Synthetic procedures used to prepare the compounds used in this work: (a) DIC/HOBT/DCM/BocNH-(CH₂)₂-NH₂; (b) TFA/DCM; (c) PhSH/BF₃·Et₂O; (d) bromoethanol/NIS/TfOH; KSCN/DMF; AcOH/Zn; (e) Cs₂CO₃/DMF; (f) NaOMe/MeOH.

glass slides covered with monolayers of the ferrocene derivative **L-1** which were analyzed by SIMS-TOF and XPS.¹⁰ Presence of **L-1** on the glass slides was confirmed by the relative abundance of carbon, oxygen and iron atoms on the chips (Fig. S1 and S3, ESI†). In the final step, the freshly prepared ferrocene monolayers were immersed in solutions of the β -CD derivatives, **C-2** or **C-3** (10 μ M), for 30 min at RT, and sugar coated surfaces were

formed by complexation of the ferrocene skeleton in the cyclodextrin cavity. The substrates were rinsed with deionized water to remove the uncomplexed compound and the slides were checked again by SIMS-TOF and XPS to reveal the presence of sulphur atoms in addition to those that were found on the slide before complexation, confirming the presence of **C-2** or **C-3** on the surfaces (Fig. S2 and S3, ESI†). Finally, the concentration of mannose on the slide was quantified by phenol-sulfuric acid assay.

Glass slides thus modified were used for binding different bacterial strains *via* interaction with the sugar molecules found on the slides. Three bacterial strains, differing in their sugar recognition properties, were used to assess whether the sugar functionalized glass slides could recognize the specific bacterial strain. We used *E. coli* ORN 178 having a mannose receptor (FimH), its mutant strain *E. coli* ORN 208 and *P. aeruginosa* having a fucose binding PIM-2 receptor. First, bacteria were grown at 37 °C and a standard growth curve was recorded at OD₆₀₀. Glass slides coated with **L-1**, **C-1/L-1**, **C-2/L-1** and **C-3/L-1** were dipped in a solution with a concentration of approximately 10⁸ bacteria for 30 minutes. The slides were then washed several times with distilled water to remove unbound or weakly bound bacteria and finally, bound bacteria were treated with DAPI or FITC or rhodamine and imaged microscopically. Glass slides coated with **L-1**, **C-1** or **C-3** were not found to bind bacteria of *E. coli* strain. However, **C-2** functionalized glass slides revealed a strong cluster of ORN 178, which could not be removed even upon vigorous rinsing with water. On the other hand, all glass slides with ORN-208 did not show binding. These results demonstrate selective and specific carbohydrate-protein interactions in bacterial recognition. Similar experiments were carried out using a *P. aeruginosa* strain. As expected, specific fucose mediated aggregation of bacteria was clearly observed (Fig. 2) which corroborates the selectivity of the binding assay.

After assessing the selectivity of the platforms in bacterial recognition, we determined the sensitivity of the detection platform. Glass slides with **C-2/L-1** modified surfaces were immersed and incubated in serially diluted solutions of mannose-binding *E. coli* ORN 178 for various periods of time, and surface-bound bacteria were visualized by DAPI staining. We found that as little as 10⁵ bacterial cells bound to the surfaces could be observed by fluorescent staining of the clusters (Fig. 3 and Fig. S4, ESI†).¹¹ It is interesting to note that this is also the minimum number of bacterial cells needed for formation of each bacterial colony.⁶

In order to assess the feasibility of the system to serve as a biosensor, it was crucial to demonstrate the reversibility of the system, *i.e.*, to prove that the slides could be used for more than one binding event and regeneration-generation cycles. This was done by utilizing the high binding constant of the β -CD-adamantane complex (5.7×10^4 M⁻¹) as compared to that of ferrocene (9.9×10^3 M⁻¹).⁷ When glass substrates coated with **C-2** sugar bound to ORN 178 bacteria were incubated in a solution of adamantane carboxylic acid (0.1 mM) for 5 minutes, the sugar was repelled from the slide. The adamantane skeleton replaced ferrocene in the cavity of the β -cyclodextrin skeleton and detached it from the glass surface. After washing the glass

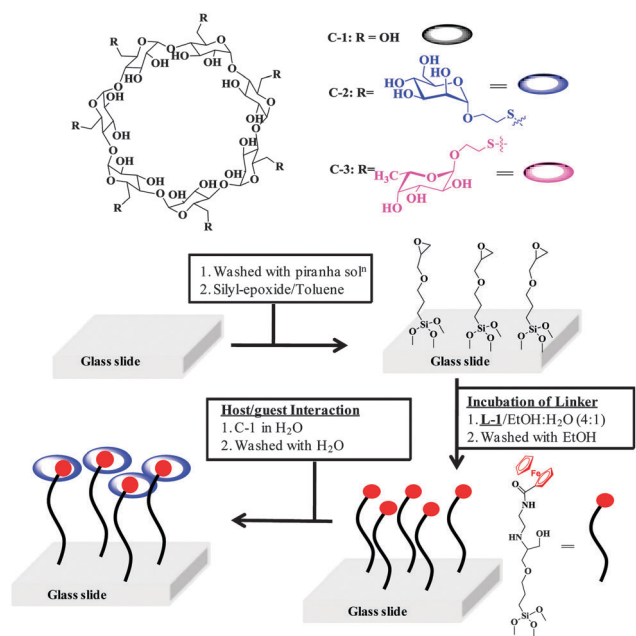


Fig. 1 Molecular structures and schematic representation of different steps to immobilize sugar-modified β -CD supramolecular scaffolds onto glass slides.

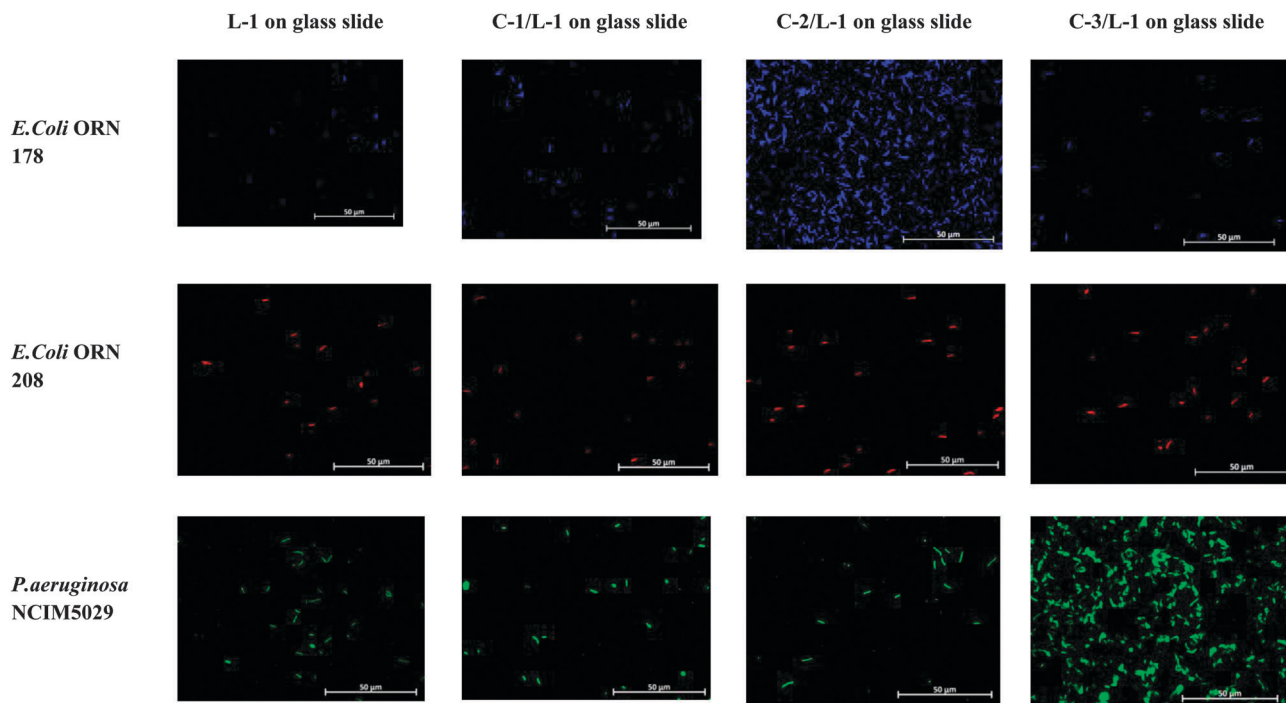


Fig. 2 Representative images of bacterial adhesion on different sugar substrates coated onto glass slides.

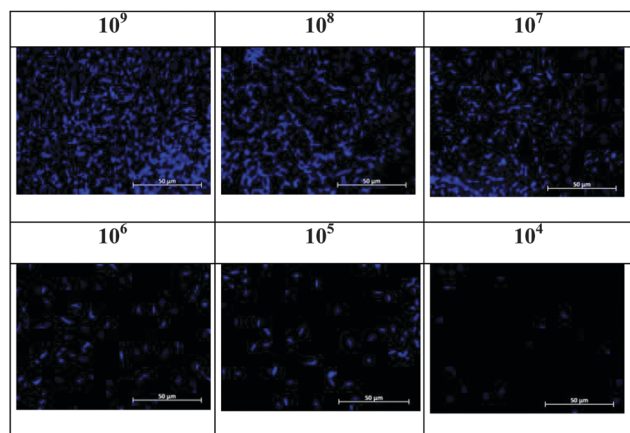
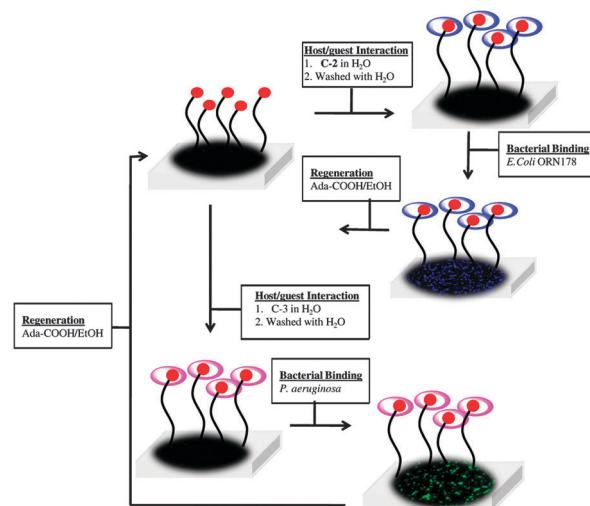


Fig. 3 Detection limit for the staining of mannose binding *E. coli* on the C-2/L-1 surface. The number of bacteria used in the incubation mixture is shown above the images.

substrate with PBS, fresh ferrocene modified surfaces were obtained. Visualizing through fluorescence microscopy revealed no bacterial aggregation and corroborated the regeneration process. When the same slide was incubated again in solutions of C-2 or C-3 for 30 minutes, sugar respective bacterial aggregations were observed on the glass slides (Scheme 2, Fig. 4). Several cycles (5) of degeneration–generation processes were performed and the reproducibility was found to be excellent.

We have demonstrated that CD-based glycodendrimers placed on glass substrates by formation of complexes, controlled by host–guest interactions, produced a platform for detection of specific types of bacteria, such as *E. coli* and *P. aeruginosa*, with



Scheme 2 Schematic describing the regeneration process of sugar coated glass slides and subsequent interaction with *E. coli* (ORN 178) and *P. aeruginosa* – regeneration–generation cycles.

high sensitivity. The interactions that control the binding are carbohydrate–protein interactions and the sensitivity is determined by the sugar moieties placed on the slide. The assay is reversible; and regeneration of the glass substrate has been demonstrated. The platform described here can be used for continuous bacterial sensing and could potentially be used as a detection method of choice in point-of-care testing. The possibility of producing microarrays with multitude types of carbohydrate on a single glass substrate for high-throughput detection of several bacteria on a single platform is currently under study.

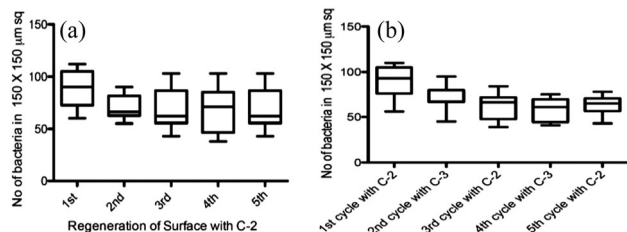


Fig. 4 Quantitative analysis of bacterial adhesion on regenerated surfaces: (a) *E. coli* ORN 178 was incubated on **C-2/L-1** glass slides for 30 min (1st cycle). After imaging, the slide was dipped in 0.1 mM of adamantane carboxylic acid solution for 5 min to remove host-guest and bacterial adhesion, later the slide was dipped in **C-2** (10 μM) for 30 min and ORN 178 for another 30 min and imaged again (2nd cycle). The platform was regenerated five times using this reiterative process. The number of adhered bacteria in a 150 × 150 μm sq area was counted manually ($n = 9$); (b) *E. coli* ORN 178 and *P. aeruginosa* binding on **C-2** and **C-3** modified surfaces was successively regenerated and bacterial adhesion in a 150 × 150 μm sq area was counted manually ($n = 9$). Note: all experiments were carried out with equal number of bacteria (10^7) and equal concentration of **C-2** or **C-3** respectively (10 μM).

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