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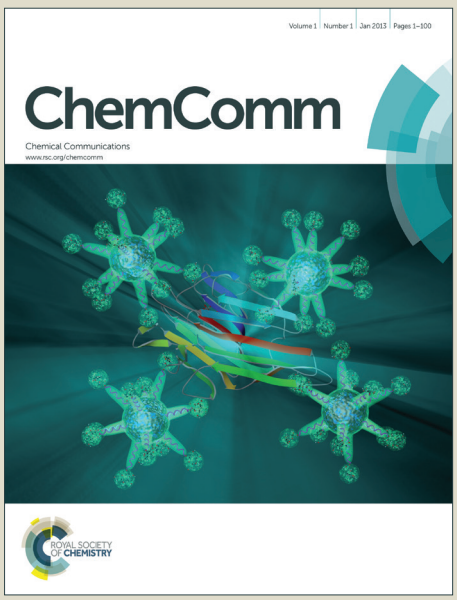

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Supramolecular Scaffolds on Glass Slides as Sugar Based Rewritable Sensor for Bacteria

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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] Major reasons for 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 using DNA based PCR assay and protein-based ELISA[3] 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 oligosaccharide 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 paper 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 molecules[7] 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 adamantane linker is the difference in the strength of the host-guest complexation[7], which yield 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]

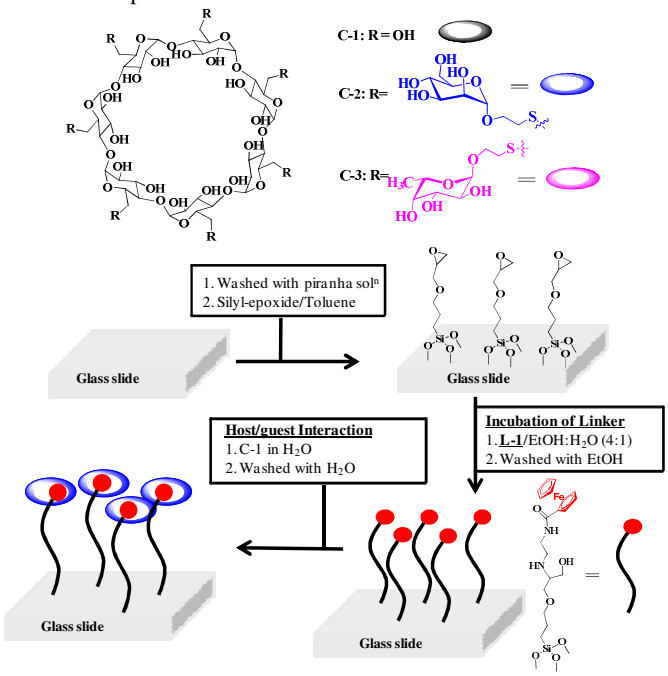


Figure 1. Molecular structures and schematic representation of different steps to immobilize sugar-modified *β*-CD supramolecular scaffolds onto glass slides.

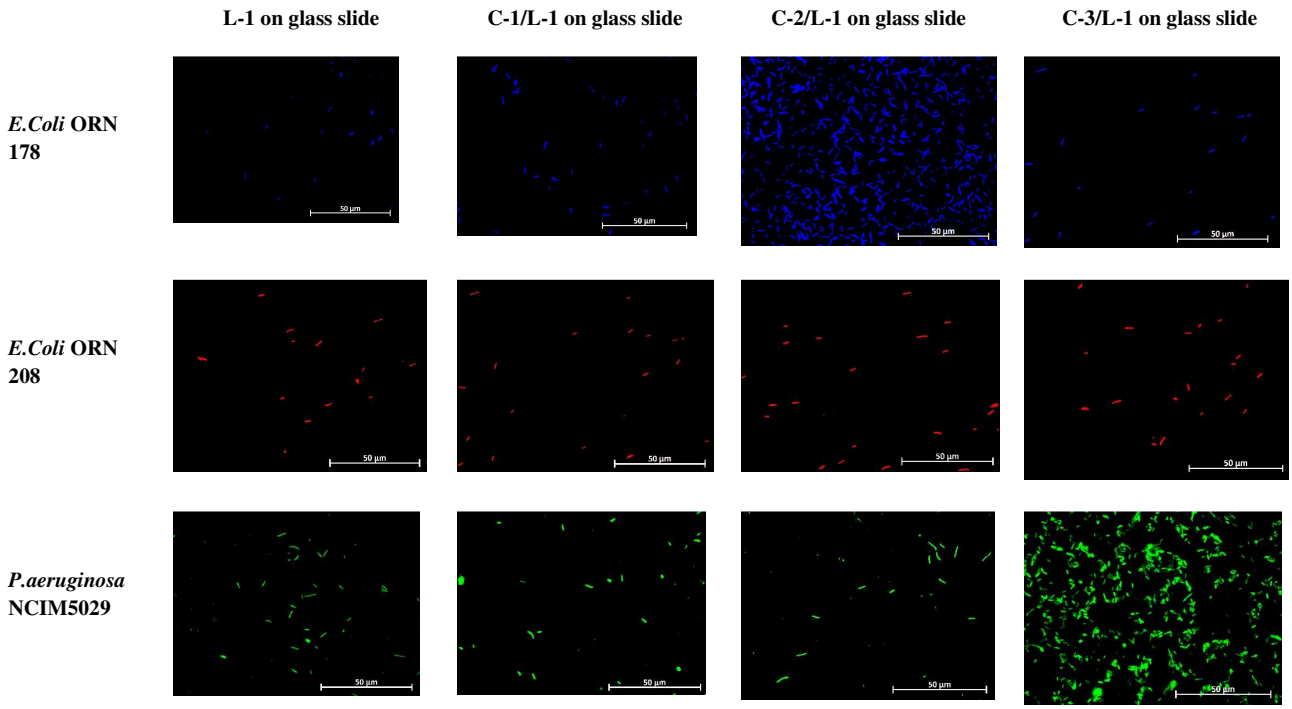
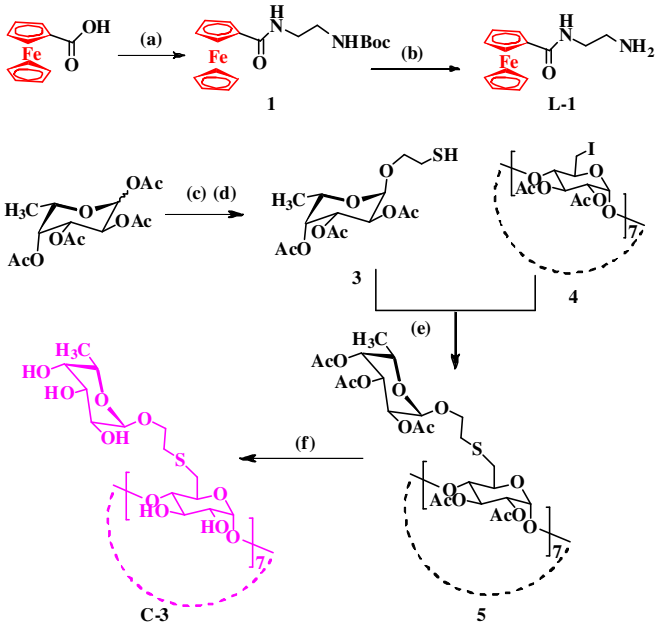


Figure 2. Representative images of bacterial adhesion on different sugar substrates coated onto glass slides.



Scheme 1. Synthetic procedures used to prepare the compounds used in this work: (a) DIC/HOBT/DCM/BocNH-$(CH_2)_2$-NH_2; (b) TFA/DCM; (c)PhSH/BF_3.Et_2O; (d) Bromoethanol/NIS/TfOH; KSCN/DMF; AcOH/Zn; (e) Cs_2CO_3/DMF; (f) NaOMe/MeOH.

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. 1x1 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 were dipped in a solution of **L-1** (0.02 mM) in ethanol for 24 h. Finally the substrates were rinsed with ethanol to remove excess ferrocene derivative and to remove residual epoxide groups (Fig 1). The process yielded 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 & S3). In the final step, the freshly prepared ferrocene monolayers were immersed in solutions of the *β*-CD derivatives, **C-2** or **C-3** (10 μM), for 30 mins 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 & S3). Finally, concentration of mannose on 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 specific bacterial strain. We used *E. coli* ORN 178 having mannose receptor (FimH), its mutant strain *E. coli* ORN 208 and *P. aeruginosa* having fucose binding PIM-2 receptor. First,

bacteria were grown at 37 °C and a standard growth curve was recorded at OD_{600}. 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 concentration of approximate 10^8 bacteriae 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 was not 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 with 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.

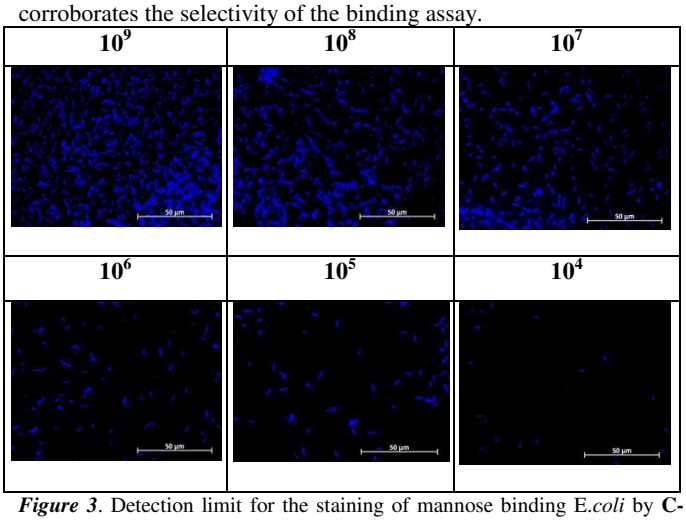
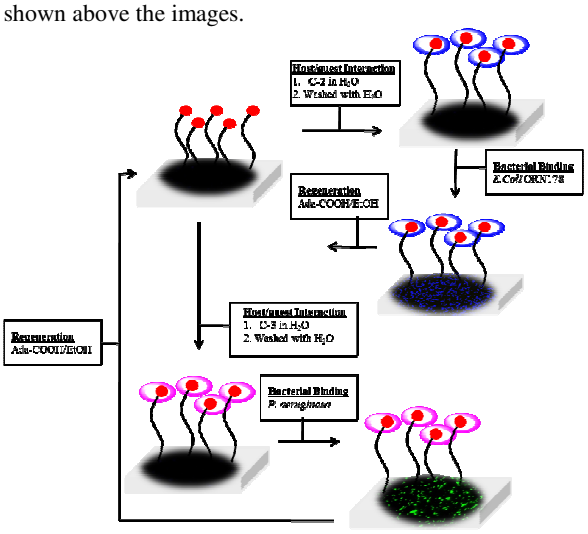


Figure 3. Detection limit for the staining of mannose binding E.*coli* by **C-2/L-1** surface. Number of bacteria used in the incubation mixture is shown above the images.



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.

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 have found that as little as 10^5 bacterial cells bound to the surfaces could be observed by fluorescent staining of the clusters (Fig 3 & S4). [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 β-CD- adamantane complex (5.7 X 10^4 M^{-1}) as compared to that of Ferrocene (9.9 X 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 of the glass 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.

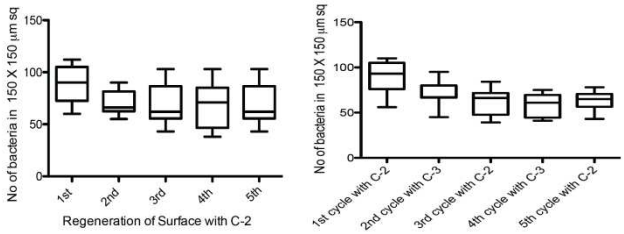


Figure 4. Quantitative analysis of bacterial adhesion on regenerated surfaces: (a) *E. coli* ORN 178 was incubated in **C-2/L-1** glass slides for 30 min (1st cycle). After imaging, the slide was dipped in 0.1 mM of adamantane carboxylic acid solution for 5 min to remove host-guest and bacterial adhesion, 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 X 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 surface were successively regenerated and bacterial adhesion in 150 X 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).

Conclusions

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 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 bacteriae on a single platform is currently under study.

Notes and References

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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