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Assessing the Effect of Different Shapes of Glyco-gold nanoparticles on Bacterial Adhesion and Infections

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Achieving selective and sensitive carbohydrate-protein interactions (CPIs) using nanotechnology is an intriguing area of research. Here we demonstrate that the different shapes of gold nanoparticles (AuNPs) functionalized with monosaccharides tune the bacterial aggregations. The mechanism of aggregation revealed that the large number of surface interaction of rod shaped mannose-AuNPs with E.coli ORN 178 compared with spherical and star-shaped AuNPs exhibited higher avidity and sensitivity. Moreover, such sensitive binding can be used for effective inhibition of bacterial infection of cells.

Carbohydrate-protein interactions (CPIs) are one of the most important and major events on cell surfaces.1 Basic components involved in the interaction are the cell surface glycans, which demonstrate a sensitive and selective cis/trans binding with protein counterparts. However, due to weak CPIs, nature facilitates multivalency to target specific proteins. Recently, extensive efforts have been directed toward mimicking these bio-events by replicating the multivalent scaffolds.2 However, to fully understand CPIs, it is important to quantify binding affinity with different size, shape, orientation, and local concentration of the sugars.3 Varieties of synthetic multivalent templates such as cyclodextrin,4 calixarenes,5 polymers,6 dendrimers,7 supramolecular complexes,8 have been extensively used to decorate sugars in particular topology to target CPIs. These synthetic templates offer controllability and reproducibility, but the drawbacks include production methods that sometimes require complex synthetic conditions. Another class of templates for multivalent glyco-probes are nanoparticles (NPs), such as gold, silver, iron and CdSe NPs.9 These templates are more rigid and easy to synthesize in large quantities in different sizes, shapes and orientations and can also be easily decorated chemically and biologically.10 Among these NPs, AuNPs are more attractive for their different shapes (rod, sphere, star, cubic and spindle), surface resonance band and ease of characterization by UV-visible and transmission electron microscopy (TEM).10 Moreover, they are less toxic compared to quantum dots. Penadés and co-workers used AuNPs to decorate more than one sugars to target HIV, bacteria, modulation of immune responses, and CPIs.11 Similarly, enormous effort have been expended by the group of Lin, Wu, Tseng and others to develop gold nanodots and nanoclusters for the sensitive and selective detection of E.coli.12 However, in all these studies, the shape of AuNPs were kept constant to validate the binding with bacteria, cells or organs, limiting the assessment of the role of different shapes of nanoparticles involved in specific CPIs. The latter is of fundamental importance for understanding the CPIs and developing new biomaterials. Various targeting units, such as antibodies, peptides, aptamers have been functionalized on different shapes of AuNPs to enhance their specificity for tumors, immune responses and biosensing processes.13 However, a systematic investigation of shape dependent CPIs with the same volume and sugar density and its potential applications have not been reported.

Scheme 1. Synthesis of mannose and galactose modified linker: (a) BF3·Et2O/DCM; (b) Thioaceticacid/AlIBN/dioxane/60°C; (c) NaOMe/MeOH

Herein, we report the use of glyco-AuNPs in bacterial recognition and inhibiting bacterial infection. Three different shapes (rod, sphere and star) of gold nanoparticles coated with mannose and galactose sugar substrates and PEG were used to quantify the binding affinity with E. coli. To profile the potential applications of the shape dependent CPIs, inhibition of E. coli infection of HeLa cells was quantified.
Scheme 2. Synthesis of AuNPs.

The monovalent mannose- and galactose-modified linkers (7 and 8) were synthesized by using the slightly modified procedure from the protocol reported in the literature. Briefly, the conjugation of triethylene glycol with 11-bromoundec-1-ene, followed by glycosylation with per-acetylated mannose or galactose yielded compound 3 or 4. These compounds were further treated with thioacetic acid in the presence of azo-isobutyronitrile (AIBN), followed by deacetylation with NaOMe to yield compound 7 or 8. (Scheme 1).

Rod AuNPs coated with cetyltrimethylammonium bromide (CTAB) were synthesized via a seeding growth method as reported previously (Scheme 2). Synthesis of star shaped AuNPs was carried out by biocompatible surfactant free method and spherical shaped AuNPs by reducing chloroauric acid with sodium citrate. Finally, sugar encapsulation of AuNPs was carried out by ligand exchange process.

Table 1. Physical characteristics of AuNPs

<table>
<thead>
<tr>
<th>Particle</th>
<th>Diameter(s) (nm)</th>
<th>$\lambda_{max}$ (nm)</th>
<th>$\zeta$-potentials (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod</td>
<td>45.6 ± 3.5 × 11.3 ± 0.5</td>
<td>798</td>
<td>34.4± 0.3</td>
</tr>
<tr>
<td>Sphere</td>
<td>18.5 ± 2.5</td>
<td>525</td>
<td>-24± 0.2</td>
</tr>
<tr>
<td>Star</td>
<td>40.6 ± 3.1 × 17.1 ± 0.9</td>
<td>715</td>
<td>-29± 1.4</td>
</tr>
<tr>
<td>Man-rod</td>
<td>45.9 ± 3.2 × 11.5 ± 1.7</td>
<td>799</td>
<td>-10.7± 0.5</td>
</tr>
<tr>
<td>Gal-rod</td>
<td>45.2 ± 2.2 × 11.8 ± 0.7</td>
<td>802</td>
<td>-17.3± 0.8</td>
</tr>
<tr>
<td>PEG-rod</td>
<td>45.9 ± 3.1 × 11.3 ± 1.5</td>
<td>798</td>
<td>-30.1± 0.6</td>
</tr>
<tr>
<td>Man-sphere</td>
<td>20.5 ± 1.3</td>
<td>520</td>
<td>-21.3± 0.5</td>
</tr>
<tr>
<td>Gal-sphere</td>
<td>20.5 ± 1.2</td>
<td>525</td>
<td>-17.5± 0.3</td>
</tr>
<tr>
<td>PEG-sphere</td>
<td>19.2 ± 1.8</td>
<td>525</td>
<td>-15.3± 0.2</td>
</tr>
<tr>
<td>Mann-star</td>
<td>44.2 ± 0.2 × 18.2 ± 0.9</td>
<td>725</td>
<td>-22.4± 1.1</td>
</tr>
<tr>
<td>Gal-star</td>
<td>42.9 ± 0.2 × 18.2 ± 0.7</td>
<td>725</td>
<td>-23.8± 1.3</td>
</tr>
<tr>
<td>PEG-star</td>
<td>44.6 ± 0.2 × 17.4 ± 0.9</td>
<td>725</td>
<td>-16.2± 1.6</td>
</tr>
</tbody>
</table>

The number of sugar molecules on AuNPs was quantified by using a phenol-sulfuric acid method (Table S1). As expected almost equal concentration of sugar scaffolds confirmed. Experiments were performed using two E. coli strains to validate the shape dependent bacterial adhesion. The choice of bacterial strains was based on their ability to recognize specific sugar substrates. In this study, we choose FimH-mannose interactions based on ORN 178 and ORN 208 is a mutant having no FimH receptors. Fluorescence imaging clearly showed aggregation of bacteria due to specific CPIs.

Figure 1. TEM images of (a) rod; (b) sphere; (c) star shaped gold nanoparticles

Figure 2. (a) E. coli strain ORN178 aggregation using AuNPs; (b) Quantification of bacterial aggregation in the presence of different shapes of AuNPs functionalized with mannose. Data represent the mean of ± SEM (n=3)
Our first experiment was to establish the binding selectivity. In this experiment, we incubated different shapes and sugar conjugated AuNPs with ORN 178 and ORN 208 for 1 h in PBS solution. After that cells were centrifuged and the aggregates formed by the bacteria were imaged and quantified. As expected, the maximum number of aggregation was observed with mannose coated AuNPs compared to galactose and PEG counterparts (Fig. 2a). On the contrary, no aggregation formation was seen in ORN 208 in any of AuNPs (Fig S4). Thus, the difference in specific vs nonspecific binding was highly dependent on the sugar scaffold compared to the shape of the AuNPs. Although all three different shapes of AuNPs contained same amount of sugar, they apparently differ in three important physical properties such as: (1) contact area of the NPs with respect to external stimuli; (2) rotational volume availability and; (3) aspect ratio. Thus, we hypothesize that specific shape might induce sensitive bacterial adhesion.

![SEM images of mannose functionalized AuNPs (Man-AuNPs) with E. coli ORN 178 stain: (a)-(d) Rod; (b)-(e) Sphere; (c)-(f) Star; (g) Quantification of attachment of Man-AuNPs on the surface of bacteria. Data represent the mean number of nanoparticles on the surfaces of bacteria (n =10); (h) Man-AuNPs bound to the surface of bacteria according to the surface availability; Conc of AuNPs = 0.8 µg/ml (n=5). (Note: While quantifying the bound AuNPs and area, the surface of the bacteria was assumed as flat surface)](image)

To demonstrate the shape dependent bacterial adhesion, experiments were performed using different concentrations of rod, sphere and star shaped Man-AuNPs (Fig. S5). The dose-response bar graph of different shapes is presented in Fig 2b. Two groups of interactions could be identified in terms of their aggregation. The first group, composed of sphere and star shape AuNPs, gave a detection limit of 17 ± 2 µg/ml and 14 ± 2 µg/ml respectively with known number of ORN 178 E.coli stain. The second group, composed of rod-shaped AuNPs, gave a detection limit of 0.03 ± 0.01 µg/ml at the same conditions. The observed ~80-fold difference in the detection of bacteria by rod AuNPs could be attributed to several factors: 1) aspect ratio, which could increase specific attachment of particles on bacterial cell surfaces; 2) the surface effective availability for AuNPs binding; 3) self-assembly of AuNPs to amplify the specific interaction. To further validate the above results, SEM images were done at the lowest concentration (0.8 µg/ml) of AuNPs treated bacteria (Fig. S7-S9). As expected, the relative amount of mannose rod AuNPs involved in effective CPIs is higher than sphere and star counterparts. This might be because of the large number of rod AuNPs interaction with E.coli surface compared to star or spherical counterparts (Fig. 3a-b). On close examination of the rod shaped AuNPs, we observed head-to-head self-assembly of on the surface of the bacteria (Fig S7). In contrast, star and spherical AuNPs under the same conditions resulted monodispersed and very few aggregations, indicating that the aspect ratio of AuNPs are crucial for self-assembly and to improve the sensitive interactions (Fig 3c-f) We also quantified the number of nanoparticles adhered on a specific µm² area of the bacteria (Fig 3g). The binding density of rod AuNPs was found nearly 2.5 fold higher than that of star and spherical shaped AuNPs. This confirmed that the rod AuNPs occupied more surface area on bacteria than that of star and sphere AuNPs, which directly modulate the binding affinity. All these results correlate to mathematical model and flow chemistry experiment proposed by Kohlar et al. Finally, we quantified the number of AuNPs actively bound on the surface of the bacteria. We observed that spherical AuNPs occupied nearly 10 and 4 fold less surface area of the bacteria compared to rod and star AuNPs, indicating that rod AuNPs exhibit higher avidity toward the bacteria (Fig 3h).

To demonstrate potential applications of shape dependent CPIs, HeLa cells, which have been known to express high mannose on the cell surface, were infected with a known amount of DAPI stained ORN 178 E. coli. We examined infection caused by the bacteria in the presence three different shapes and five different concentrations (12, 25, 50, 100 and 200 µg/ml) of mannose-AuNPs. After 1 h of incubation, both bound and unbound bacteria were separated and quantified by measuring the fluorescence intensity of DAPI. Results obtained from different shapes showed that the percentage of inhibition (unbound bacteria) caused by rod AuNPs was approximately six-fold more than that of star and spherical AuNPs (Fig 4). To confirm that the inhibition effect of mannose-rod AuNPs is indeed anchored by specific CPIs, ConA and PNA (10 µl of 0.1 mg/ml) were added to mannose-AuNPs before mixing with bacteria and HeLa cells. Due to specific CPIs between mannose-ConA most of the cells got infected, whereas in the presence of PNA lectin, which is specific to galactose sugars showed effective inhibition of cell-bacterial interactions. These results confirmed that the shape of the AuNPs and specific CPIs fine tune the inhibition properties.

![Figure 3. (a-f) SEM images of mannose functionalized AuNPs (Man-AuNPs) with E. coli ORN 178 stain: (a)-(d) Rod; (b)-(e) Sphere; (c)-(f) Star; (g) Quantification of attachment of Man-AuNPs on the surface of bacteria. Data represent the mean number of nanoparticles on the surfaces of bacteria (n =10); (h) Man-AuNPs bound to the surface of bacteria according to the surface availability; Conc of AuNPs = 0.8 µg/ml (n=5). (Note: While quantifying the bound AuNPs and area, the surface of the bacteria was assumed as flat surface)](image)

![Figure 4. Inhibition of E. coli infection of HeLa cells: (a) percentage of bound bacteria; (b) percentage of unbound bacteria. Quantification was done with respect to the DAPI fluorescence intensity, which](image)
corresponds to the concentration of the bacteria taken for the above experiment (control). Fluorescence intensity was calculated from the average of three independent experiments.

**Conclusions**

In summary, we have demonstrated that shape is one of the factor in establishing sensitive carbohydrate-protein interactions. Data from the bacterial adhesion and HeLa cells infection studies showed that the rod-shaped AuNPs functionalized with mannose had substantial sensitivity compared to that of star-shaped and spherical shaped AuNPs. Factors such as self-assembly and effective surface contact are critical for sensitive adhesion. In a more general perspective, blockage of cell surface contact are critical for sensitive adhesion. In a more general perspective, blockage of cell surface contact are critical for sensitive adhesion. In a more general perspective, blockage of cell surface contact are critical for sensitive adhesion.

**Acknowledgements**

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