Carbohydrate capped silicon nanoparticles including galactose, glucose, mannose, and lactose have been successfully developed as targeting and monitoring agent for drug delivery. They show reduced toxicity both in vitro and in vivo compared to amine terminated silicon nanoparticles, and can be taken up more readily by cancerous cells than noncancerous cells.
Synthesis of Carbohydrate Capped Silicon Nanoparticles and their Reduced Cytotoxicity, In Vivo Toxicity, and Cellular Uptake

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Keywords: cancer cells, carbohydrates, nanotoxicity, silicon nanoparticles, uptake

ABSTRACT: The development of smart targeted nanoparticles (NPs) that can identify and deliver drugs at a sustained rate directly to cancer cells may provide better efficacy and lower toxicity for treating primary and advanced metastatic tumors. Obtaining knowledge of the diseases at the molecular level can facilitate the identification of biological targets. In particular, carbohydrate-mediated molecular recognitions using nano-vehicles are likely to increasingly affect cancer treatment methods, opening a new area in biomedical applications. Here, silicon NPs (SiNPs) capped with carbohydrates including galactose, glucose, mannose, and lactose have been successfully synthesized from amine terminated SiNPs. The MTT analysis shows an extensive reduction in toxicity of SiNPs by functionalizing with carbohydrate moiety both in vitro and in vivo. Cellular uptake has been investigated with flow cytometry and confocal fluorescence microscope. The results show the carbohydrate capped SiNPs can be internalized in the cells within 24 h of incubation, and can be taken up more readily by cancer cells than noncancerous cells. Moreover, these results reinforce the use of carbohydrates for the internalization of a variety of similar compounds into cancer cells.

1. Introduction

Every year, millions of people’s lives worldwide are affected by a complex group of diseases known as cancer. Even though traditional chemotherapies that indiscriminately act against actively dividing cells have been identified as one of the most effective methods of cancer therapy, chemotherapeutic agents exhibit poor specificity in reaching tumor tissue and are often restricted by dose-limiting toxicity. To overcome these major drawbacks of chemotherapy, targeted drug delivery, and controlled drug release technology may provide a more efficient and less harmful solution, as targeted therapies specifically interfere with those molecules essential for tumor growth and progression.[1] Targeted therapies include monoclonal antibodies and small molecule inhibitors targeting tumor specific markers. It is well known that tumors express particular molecular signatures that discriminate them from healthy tissues, providing a rich source of potential targets. However, there are some methodological weakness to this general approach. For example, the identification of tumor specific markers is a time consuming process. Validating any application requires that patients must be stratified prior to treatment to ensure that the appropriate targets are present in each case. As such these treatments are expensive, for example colorectal cancer targeted therapies can cost several thousand dollars per month.[2] Therefore, as people live longer, with accompanying improvements in early detection rates, the financial burden of providing targeted cancer therapies is expected to rise substantially. Furthermore, tumor cells have high tendencies to mutate, which changes their antigenic modifications leading to negative results. A good example of this is the multiple resistance mechanisms observed in targeting the constitutively
active, pro-proliferative Bra\textsuperscript{V600E} mutation in melanoma.\textsuperscript{[1–3]} A possible alternative to tumor-specific targeting using over-expressed oncogenes is to take advantage of other molecules present on the surface of cells, such as cell surface carbohydrates.

Carbohydrates are attractive targets for receptor-mediated interaction and in particular glycol-conjugates, which play important roles in cancer development and metastasis. Many types of mammalian cells are covered with a dense layer of carbohydrates known as the glycol-calyx, in which carbohydrates are bound to proteins and lipids to form glycoproteins, proteoglycans, and glycolipids. These naturally occurring glycol-conjugates play an important role in the process of cell–cell interactions and cell–cell communication that is vital for physiological and pathological processes.\textsuperscript{[30–32]} As one of the most common cell–surface ligands, carbohydrates can directly initiate the initiation of many medicinally important physiological processes where they are involved in a wide variety of cellular events,\textsuperscript{[12–14]} including inflammatory and immunological responses,\textsuperscript{[15–17]} tumor metastasis,\textsuperscript{[18]} cell–cell signaling,\textsuperscript{[19]} apoptosis, adhesion,\textsuperscript{[20]} bacterial and viral recognition,\textsuperscript{[20,21]} and anticoagulation.\textsuperscript{[22]}

The biological roles of carbohydrates as signaling effectors and recognition markers are associated with specific molecular recognition in which proteins\textsuperscript{[23]} or other carbohydrates\textsuperscript{[24]} are involved. This characteristic led to the identification of tumor-associated carbohydrate molecules,\textsuperscript{[19,25]} which has greatly improved the development of carbohydrate-based anticancer vaccine studies.\textsuperscript{[26]} However, the understanding of carbohydrate-binding properties of tumors is not well defined. Cancer cells can interact with the extracellular matrix in their microenvironment through endogenous receptors binding with cognate carbohydrates.\textsuperscript{[26,27]} These interactions vary, depending on the physiological state of the cells, as supported by histological studies of tumor tissues.\textsuperscript{[18,28]} Therefore, the ability to characterize and distinguish carbohydrate binding profiles of a variety of cells can expedite both the mechanistic understanding of their role in disease development and the expansion of diagnostic and therapeutic tools.\textsuperscript{[10–12]}

When nanoparticles (NPs) are functionalized with ligands such as antibodies, proteins or peptides, oligonucleotides or carbohydrates, they become excellent vehicles for biological applications at the cellular and molecular level.\textsuperscript{[13]} However, several features need to be fine-tuned including ligand density, particle diameter, surface charges, magnetic, electronic or optical properties, stability, and targeting specificity. Nanomaterials can serve as promising platforms for displaying carbohydrates for biological recognition. Due to the smaller sizes of NPs compared to their micrometer-sized counterparts, NPs have much larger surface areas, which can enable higher capacity in receptor binding. In addition, multiple carbohydrate ligands can be immobilized onto one NP, which can potentially enhance the weak affinities of individual ligands to their binding partners.

Silicon NPs (SiNPs) hold prominent interest in various fields of biomedical research including imaging, detection, sensing to drug delivery and new therapeutic uses for a variety of diseases including cancer.\textsuperscript{[14,15]} This is in addition to the electronic, magnetic, and optical properties exhibited by these NPs. SiNPs or quantum dots have size dependent tunable light emission, bright luminescence and stability against photobleaching compared to organic fluorescent dye molecules, which makes them ideal tools for fluorescence imaging. All these properties have enabled SiNPs to be used as fluorescent cellular markers in a number of diagnostic and assay roles.\textsuperscript{[36,37]} Moreover, when comparing with heavy metal and other types of semiconductor quantum dots, SiNPs exhibit low inherent toxicity.\textsuperscript{[37–40]}

Herein, we explore the possibility of using glyco-conjugated SiNPs to detect various cancer cell types on the basis of the more physiologically related carbohydrate–receptor interactions. We have focused on SiNPs functionalized with carbohydrates that play key roles in molecular recognition processes, rather than those where carbohydrates mainly function as NP stabilizing agents.\textsuperscript{[41–43]} The information obtained on the physiologically relevant carbohydrate–receptor interaction will not only enhance our understanding of the role carbohydrates play in cancer, but also guide the development of potential therapeutics such as novel contrast agents for tumor detection.

2. Results and Discussion

2.1. Stability of Carbohydrate Capped SiNPs in Biological Media

A significant challenge in the use of NPs for biomedical applications is to retain their stability in biologically associated environments. In order to achieve this, NPs have to be hydrophilic and maintain superior stability in biological media. For advanced biomedical applications of NPs (e.g., in vivo diagnostics and therapy), additional requirements such as minimization of nonspecific uptake by reticuloendothelial systems must be imposed in order to achieve long blood circulation time and high diagnostic or therapeutic efficiency.\textsuperscript{[44]} To investigate the in vivo effects of NPs in the circulation, and to measure the effects of NPs on different cell types in vitro, NPs have to be dispersed in physiological solutions. However, particles which are dispersed in solutions containing physiological salt concentrations and pH values can form micrometer-sized coarse agglomerates.\textsuperscript{[45–47]} Coarse agglomerates of NPs have been shown to exert different biological effects compared to well-dispersed nanoparticles.\textsuperscript{[48–50]} Moreover, aggregation can induce toxicity both in vitro and in vivo models.\textsuperscript{[51]} Therefore, it is important to investigate the effect of biological media on carbohydrate capped SiNPs.

Dynamic light scattering (DLS) was used to assess the stability of carbohydrate capped SiNPs in biological media by monitoring the variation of the average hydrodynamic size of the NPs. For long-term stability testing, 0.1 mL solution of carbohydrate capped SiNPs was incubated in 1 mL of Dulbecco’s modified eagle’s medium (DMEM) with 10% FBS, Roswell Park Memorial Institute (RPMI) with 10% FBS and 0.1 X Marc’s Modified Ringers (MMR) as a function of time. The average hydrodynamic diameter was measured at various time points. Figure S1, Supporting Information, and Table 1 show the stability of carbohydrate capped SiNPs by DLS in DMEM, RPMI,
Table 1. Diameter of carbohydrate capped SiNPs by DLS in DMEM, RPMI, and MMR media.

<table>
<thead>
<tr>
<th>Carbohydrate capped SiNPs</th>
<th>DMEM media DLS size[mm]</th>
<th>RPMI media DLS size[mm]</th>
<th>MMR media DLS size[mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>12 h</td>
<td>24 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Gal SiNPs</td>
<td>10 ± 1.0</td>
<td>10 ± 1.0</td>
<td>15 ± 1.5</td>
</tr>
<tr>
<td>Man SiNPs</td>
<td>11 ± 1.0</td>
<td>13 ± 1.0</td>
<td>14 ± 1.0</td>
</tr>
<tr>
<td>Glu SiNPs</td>
<td>13 ± 1.0</td>
<td>13 ± 1.0</td>
<td>13 ± 1.0</td>
</tr>
<tr>
<td>Lac SiNPs</td>
<td>14 ± 1.5</td>
<td>14 ± 1.5</td>
<td>16 ± 1.0</td>
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</tbody>
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The stability results suggest that carbohydrate capped SiNPs as they did not interact with the protein, which is contained in the DMEM and RPMI media, and were stable. These stability results suggest that carbohydrate capped SiNPs can serve as an important platform to be used for in vivo study as well as for other biological applications.

2.2. Toxicity of Carbohydrate Capped SiNPs

2.2.1. Cytotoxicity

The effect of carbohydrate capped SiNPs on the cytotoxicity of cells was determined by MTT assay. Potential toxicity was examined in three cell lines: AS49 (Human lung carcinoma), HHL-5 (human immortalized hepatocytes) and MDCK (normal kidney epithelium cells). Cells were plated in 200 µL of complete culture medium containing 50, 300, 500, 700, and 1000 µg mL−1 concentrations of carbohydrate capped SiNPs in 96-well plates for 72 h.

The results of the MTT assay, as a measure of cell viability following 72 h exposure to carbohydrate capped SiNP, are shown in Figure 1, with amine-terminated SiNPs as a control in the three cell lines tested. It is observed that even at a higher particle concentration, i.e. 1000 µg/mL, cell viability is high for all the carbohydrate capped SiNPs (often >95%) compared to amine-terminated SiNPs, which induced toxicity at a concentration of 200 µg mL−1 and above. This suggests that the carbohydrate capping the SiNPs reduces cytotoxicity in this concentration range. The amine-terminated SiNPs induction of cytotoxicity at 200 µg mL−1 was confirmed by calculating IC50 (Figure S4, Supporting Information). As one can see from Figure 1, cell proliferation is not affected by increasing carbohydrate capped SiNP concentration in all the cell lines tested, which suggests the carbohydrate capped SiNPs did not cause any cytotoxicity nor increased the proliferative capacity of mammalian cells.

2.2.2. In Vivo Toxicity Assay

To study the interaction of nanomaterials with biological systems various in vivo biological models have been proposed which can provide a route with which to assess nanotoxicity. Embryos are particularly sensitive indicators of adverse biological effects on the organism. Moreover, they provide a useful platform to study the mechanism of action of adverse effects resulting from exposure to NPs. For normal embryo development, highly coordinated cell-to-cell communications...
and molecular signaling are required; any perturbations by nanomaterials will disrupt orderly embryogenesis leading to abnormal development, which can manifest as morphological malformations and embryonic death.\[^5\]

To validate our findings that carbohydrate capped SiNPs produce low to no cytotoxicity (Figure 1), we used *X. laevis* embryos to test SiNP toxicity in vivo. *X. laevis* offers several advantages as a toxicity assessment tool: large numbers of embryos with each fecundation (thousands) with a very short early development time (3 d to reach tadpole stages), external development, close homology with human genes, and less expensive husbandry/housing compared to small mammalian models.\[^6\]

In this work we have used *X. laevis* embryos as nanotoxicity assessment models for the carbohydrate capped SiNPs. Figure 2 shows the images of treated *X. laevis* embryos. The *X. laevis* embryos were exposed to carbohydrate capped SiNPs and amine-terminated SiNPs at Nieuwkoop & Faber (NF) stage 15 and fixed at NF stage 38.\[^7\] The toxicity was compared to control embryos (without NPs) shown in Figure 2a. In total 30 embryos were assessed with each NP, at three concentration levels (50, 100, and 200 \(\mu g \cdot mL^{-1}\)). They were then classified as to what effect the SiNP treatment had: dead, presence or not of abnormalities, common malformations (such as stunted development, bent spine and tail, eye deformities, gut abnormalities, edema, and blistering).

From Figure 2 it is clearly observed that carbohydrate capped SiNPs do not induce severe toxicity in the *X. laevis* embryos at the highest concentration of NPs tested. Lac capped SiNPs shown to be slightly toxic by looking at tail deformities compared to that of mannose (c) and glucose (d) capped SiNPs. Similarly, a few gal capped SiNPs exposed embryos showed bent spine suggesting some limited toxicity. Such observations were made only at the highest concentrations tested. In contrast, the lower concentrations did not show any morphological abnormalities in *X. laevis* embryos for carbohydrate capped SiNPs (Figure S6, Supporting Information). Compared to carbohydrate capped SiNPs, amine terminated SiNPs were shown to be highly toxic and resulted in the death of the embryos as shown in Figure 2 and Figure S6, Supporting Information. By studying both in vitro and in vivo toxicity it is clear that the carbohydrate capped SiNPs are nontoxic in the models tested, which highlights their strong potential for biomedical applications.
2.3. Cellular Uptake of Carbohydrate Functionalized SiNPs

In order to gain insights into how carbohydrate capped SiNPs internalize within the cells, a cellular uptake experiment was performed using confocal microscopy. Figure 3 shows the A549 cells incubated with gal capped SiNPs for 24 h compared to control cells. Counterstaining with AlexaFlour 594-phalloidin to stain F-actin shows that the carbohydrate capped SiNPs internalize within the cell’s cytoplasm showing green fluorescence. Punctate green staining in the SiNPs treated cells suggests that these nanomaterials are taken up into intracellular vesicles. Images from more mature cells are shown in Figures S7 and S8, Supporting Information.

2.4. Cellular Uptake Kinetics

To clarify and demonstrate the importance of the functionalization strategy on the SiNPs cell interaction, we performed cell uptake experiments using both cancerous and noncancerous cell lines. Flow cytometry was used to semiquantitatively measure cellular uptake of the SiNPs. The instrument gave an accumulated intensity of the SiNPs fluorescence in 10 000 cells. Therefore, the total fluorescence of particles in one cell is measured by this approach. The carbohydrate capped SiNPs were introduced to three cancerous cell lines (A549, MCF-7, and SK-Mel28) and two noncancerous, immortalized cell lines (MDCK and HHL5).

A major hurdle for cancer treatment and early cancer detection is the identification of pertinent cellular signatures to allow the differentiation of normal cells from their cancerous counterparts. This could be achieved by analysis of the respective cellular characteristics toward carbohydrate binding. This phenomenon was verified by evidence from the literature that MCF-7/Adr-res cells contain the cancer-specific galactoside binding galectins-4, 7, and 8, which are absent in noncancer cell lines. The Penades group also demonstrated that mouse melanoma cells are known to bind lactose due to the presence of galectins on the surface.

Initially, uptake efficiency of carbohydrate capped SiNPs was measured at various time points in all cell lines (Figure 4). This allowed us to quantitatively measure the uptake efficiency of carbohydrate capped SiNPs at a concentration of 200 µg mL⁻¹ in A549, MCF-7, SK-Mel28, MDCK, and HHL5 cells for 24, 48, and 72 h (Figure 4).

From Figure 4 it is observed that the carbohydrate capped SiNPs are successfully taken up by both cancer and noncancer cell lines. At 24 h, the mean number of intracellular gal, and lac capped SiNPs for A549, MCF-7, SK-Mel28 cells significantly...
The uptake efficiency of carbohydrate capped SiNPs was found to be significantly different in all three cancer cell lines at all-time point (Figure 5 and Figure S9, Supporting Information). This indicates there is some degree of carbohydrates uptake selectivity among these cell lines. Based on flow cytometry response, it is observed that the binding of gal capped SiNPs and lac capped SiNPs in cancerous cells is higher, suggesting that these cell lines have active galactose and lactose receptors. SK-Mel28 cells were found to interact with gal and lac SiNPs more efficiently. This is of special interest since it is reported that melanoma cells bind to lactose, due to the presence of galactin on the surface.[60]

Furthermore, the time dependent uptake study of the carbohydrate capped SiNPs was performed in SK-Mel28 cells at various time points. The cells were exposed to the NPs at various time points from a few hours to several days and the results quantified by flow cytometry analysis (Figure 5).

Figure 5 shows the carbohydrate capped SiNPs were internalized within 24 h in SK-Mel28 cells, but the internalization of NPs was found to be decreasing at 48 and 72 h. The optimal uptake efficiency was at 24 h, the speed of which indicates receptor mediated endocytosis. After 24 h lower uptake could be due to exocytosis process, metabolising Si to silicate, and cell division.

To further investigate the internalization mechanism of carbohydrate capped SiNPs we incubated the cells with the carbohydrate capped SiNPs at 4 and 37 °C. Traditionally, it has been proposed that active transport of molecules across the cell membrane is temperature dependent.[61] At low temperatures transport activity is strongly reduced, thus uptake of molecules could be attributed to a nonspecific diffusional entry into the cells.[62] Effects from low temperature may affect the binding of the ligand to specific cell receptors, the lateral mobility of the ligand-receptor complex,[63] the formation of necks in the clathrin coated pits,[64] and/or the transport of endocytosed material from endosomes to lysosomes.[65] Endocytosis of ligands such as transferrin, cholera toxin or some viruses has been shown to be temperature dependent,[66–68] as the ligands are able to attach to cell membranes at low temperatures, but not internalized. Recent work has shown uptake is energy dependent.[69–71] Based on this concept, carbohydrate capped SiNPs were incubated with the SK-Mel28 cells at low and normal incubation temperatures, and SiNP uptake analyzed using flow cytometry analysis (Figure 6). From Figure 6 it is clearly observed that the SiNPs incubated with cells at 37 °C were internalized, while the flow cytometry signals were at a significantly lower level for those incubated at 4 °C. Therefore, the obtained results suggest that the cellular uptake of carbohydrate capped SiNPs is likely to be energy dependent, suggesting it could be receptor-mediated.[72]

3. Conclusion

Our findings suggest that carbohydrate capped SiNPs could be useful tumor detection agents. These NPs proved to be very stable in biological media, shown by DLS measurements. The minimal toxicity of carbohydrate capped SiNPs was confirmed using both in vitro and in vivo models. The in vitro toxicity was verified by MTT assay in three types of mammalian cell lines. The carbohydrate capped SiNPs were found to have no significant toxicity at the highest concentration of 1000 µg mL⁻¹. The in vivo toxicity of carbohydrate capped SiNPs was examined...
in X. laevis embryos; the SiNPs were shown to be nontoxic for embryos up to 200 µg mL\(^{-1}\) and no death or morphological damage was observed. The results were compared with amine-terminated SiNPs, which were as expected highly toxic and resulted in the death of embryos. The obtained results suggest that the SiNPs are uniformly capped with carbohydrate molecules, which make them stable as well as nontoxic in both in vivo and in vitro models.

The uptake efficiency of carbohydrate capped SiNPs was quantified by flow cytometry. The obtained results indicated that carbohydrate capped SiNPs internalize in the cell within 24 h. The fluorescence uptake of carbohydrate capped SiNPs was quantified in both cancer and noncancerous cell lines and the cancerous cells more readily took up carbohydrate capped SiNPs than noncancerous lines, which is important in terms of developing future diagnostic tools or even drug delivery systems to tumors. The uptake of carbohydrate capped SiNPs was visualized by fluorescence and confocal microscopy. The NPs showed quick accumulation inside the cancer cells.

Our understanding of cancer cell functions, such as endocytosis, cell–matrix and cell–cell communications, can be greatly enhanced by studying carbohydrate–receptor functions as a result of carbohydrate capped SiNP utilization. In addition, such studies can help further understanding of specificity and ligand optimization, and exploitation of carbohydrate capped SiNPs for in vivo cancer detection.

4. Experimental Section

**Synthesis of Carbohydrate capped SiNPs:** Carbohydrate capped SiNPs were synthesized from amine-terminated SiNPs using a previously described method.\(^{[73, 74]}\) The acid functionalized carbohydrate derivatives (gal, man, glu, and lac) were synthesized according to the literature, followed by the isolation of pure anomers (detailed synthesis is presented in the supporting document). The gal, man, glu, and lac capped SiNPs were synthesized using their corresponding pyranosyl acid. A pyranosyl acid galactose, mannose, glucose, and lactose (30 mg, 0.078 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC 14 mg, 1 equiv, 0.085 mmol) were dissolved in dichloromethane (5 mL) and left with stirring for 2 h at room temperature. After 2 h N-hydroxysuccinimide (NHS) and freshly prepared amine-terminated SiNPs were dissolved in methanol and added into the reaction mixture: The reaction was stirred for 24 h at room temperature (Scheme 1).

In order to remove the unreacted amine-terminated SiNPs, the crude reaction mixture was washed three times with water (3 × 10 mL) and extracted into CH\(_2\)Cl\(_2\). The mixture was dried with Na\(_2\)SO\(_4\), and solvent was removed under vacuum. After washing, the reaction mixture was further ensued for deacetylation. Deacetylation of carbohydrate capped SiNPs was performed with sodium methoxide in methanol and then stirred for 30 min with Dowex 50WX-8 [H\(^+\)] resin. The byproduct urea and impurities were difficult to remove at this stage because both the byproduct and OAc-carbohydrate-capped SiNPs (gal, man, glu, and lac) are soluble in dichloromethane. Deacetylation of the reaction mixture removes the acetate group of the carbohydrate capped SiNPs and offers hydroxyl carbohydrate capped SiNPs, which are soluble in water. In order to obtain a pure product, the mixture was washed with dichloromethane to remove impurities and urea. A pinkish solid product of the pure carbohydrate capped SiNPs was obtained after deacetylation of each crude reaction mixture. This sticky solid was redissolved in water for further characterization. Physical characterisation results of synthesized SiNPs, such as size, size distribution, and Zeta-potential, are listed in Figures S10 and S11, Tables S1 and S2 of the Supporting Information.

**Dynamic Light Scattering (DLS):** DLS measurement was recorded with a Zetazizer Nano ZS (Malvern Instruments Ltd., U.K.). Carbohydrate capped SiNPs were dissolved in DMEM with 10% FBS, RPMI medium with 10% FBS and 0.1X Marc's modified ringers pH7.4 (MMR; 100 × 10\(^{-3}\) M NaCl, 2 × 10\(^{-3}\) M KCl, 2 × 10\(^{-3}\) M CaCl\(_2\), 1 × 10\(^{-3}\) M MgCl\(_2\), and 5 × 10\(^{-3}\) M HEPES) as a function of time. The hydrodynamic diameters were obtained at room temperature.

**Cell Culture:** The human-derived hepatocyte HHL5 and breast cancer MCF7 cell lines were a kind gift from Y. Bao, and the immortalized kidney MDCK cell line was a kind gift from M. Mogensen, University of East Anglia, Norwich. The...
human melanoma SK-mel28 was a kind gift from A. Chien and R. T. Moon, University of Washington, Seattle. The lung carcinoma A549 cell line was a kind gift from D. Sexton, Liverpool John Moores University, Liverpool. All lines used were regularly confirmed as mycoplasma free using a PCR-based assay.\textsuperscript{[26]} Cells were sub-cultured as previously described.\textsuperscript{[27]} Briefly, cells were incubated at 37 °C, 5% CO\textsubscript{2}/95% air and cultured in RPMI (MCFC7, A549) and DMEM (SK-Mel28, MDCK, HHL5) supplemented with 10% heat-inactivated foetal bovine serum, 2 × 10^{-3} M l-glutamine, 100 μg mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (all purchased from Life Technologies, Carlsbad, CA). Cells were never cultured beyond passage 30.

**Cell Proliferation Assay:** Cell proliferation in the presence of carbohydrate capped SiNPs was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Different cell lines were used in order to achieve a more complete study: HHL5 (immortalized human hepatocytes), A549 (lung carcinoma cells), and MDCK (Madin Darby canine kidney cells) cells were seeded in a 96-well plate for 24 h. The cells were then treated with carbohydrate capped SiNPs (gal, glu, man, and lac) at various concentrations (50, 300, 500, 700, and 1000 μg mL^{-1}) for 72 h. Afterward, normal procedure was applied as described in the previous publication.\textsuperscript{[28]} All experiments were repeated on at least three different occasions.

**Culturing Embryos:** These experiments were performed in compliance with institutional guidelines at the University of East Anglia. The local ethical review committee (according to UK Home Office regulations) has approved the research. X. laevis embryos were obtained as previously described.\textsuperscript{[29]} Embryos were incubated at 18 °C until the required stage was achieved according to the Nieuwkoop and Faber (NF) developmental staging system.\textsuperscript{[30]} Any dead embryos were removed throughout the culturing process. Embryos were cultured in 0.1× MMR supplemented with 25 μg mL^{-1} gentamycin.

**Exposure to Carbohydrate Capped SiNPs:** Live embryos were collected for exposure to SiNPs at NF stage 15 to 38, as described above. These stages of *Xenopus* were selected to assess NP toxicity as they represent a key stage in development of the embryos, through neurulation (NF stage 15), to tadpole stages (NF stage 38). Concentrations of NPs were made up using serial dilutions in 0.1× MMR. In a 24-well plate, 5 embryos per well were exposed to the varying concentrations of SiNPs in a total volume of 1000 μL per well. Embryos were incubated, exposed to SiNPs, at 18 °C until the required NF stage.

**Fixing Embryos:** Embryos exposed to SiNPs were fixed at NF stage 38. Fixing was carried out as previously described.\textsuperscript{[31]} Once the embryos reached the required NF stage they were washed in 0.1× MMR and fixed in MEMFA (0.1% MOPS [pH7.4], 2 × 10^{-3} M EGTA, 1 × 10^{-3} M MgSO\textsubscript{4}, and 3.7% formaldehyde) for 1 h at room temperature. After two washes in PBS all embryos were scored for gross phenotypic abnormalities.

**Confocal Laser Scanning Microscopy (CLSM):** HHL5 and A549 cells were seeded on 12-well plates with cover slips at a density of 3 × 10^4 cells per well and exposed to 150 μg mL^{-1} of carbohydrate capped SiNPs for 24 h. The cell were then washed twice by PBS and fixed by Paraformaldehyde solution for 10 min. To visualize the cells, they were stained with Lysotracker-Red (Invitrogen) or Texas Red-X Phalloidin (Invitrogen) according to manufacturer’s protocols. Then, a drop (approximately 2 μL) of fluorescent mounting medium (VECTASHIELD hard, Vector Labs) was added on top of the microscope slide. The cover slip in which cells were grown was turned upside down on top of the mounting medium. The slide was then dried in the fridge for ≈30 min before use. The images were taken under a confocal microscope (Zeiss LSM510 META system) using a 40X oil immersion objective lens.

**Flow Cytometry:** HHL5, A549, MDCK, MCF-7, and SK-mel28 cells were seeded on 24-well plates at a density of 3 × 10^4 cells per well and incubated at 37 °C overnight. The cells were washed with PBS and treated with 300 μg mL^{-1} of carbohydrate capped SiNPs at various time points from 1 h to 72 h. Afterward, cells were harvested by trypsinization and suspended in the medium (300 μL). The cellular uptake of SiNPs was examined by flow cytometry (Accuri C6 Flow Cytometer System) using 380 nm excitation wavelength. A total of 10 000 events were recorded for each sample. The samples were analyzed with Flowjo Software and an average of the mean of at least three different experiments (with different sets of NPs) was calculated.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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