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Targeting of Cancer Cells Using Click-Functionalized Polymer Capsules

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Drug delivery vehicles, such as polymer capsules, micelles and polymersomes, have significant potential to improve the therapeutic delivery of a range of drugs.[1] Combining these systems with targeted delivery to specific cells will considerably enhance the dose of drug delivered to diseased tissues while also minimizing potentially harmful side effects.[2] Herein, we describe a general approach for the functionalization of low fouling, nanoengineered drug carriers with antibodies (Ab) using click chemistry. We demonstrate that Ab-functionalized capsules bind specifically to cancer cells expressing the complementary antigen in vitro. This precise targeting occurs even when the target cells are less than 0.1% of the total cell population.

Antibodies are an important class of targeting molecule as they exhibit high specificity and selectivity for antigens expressed by specialized cells. The humanized A33 monoclonal antibody (huA33 mAb) has attracted considerable clinical attention due to its specific localization in primary and metastatic colorectal cancer in humans, and the expression of the A33 antigen on >95% of primary and metastatic colorectal cancers.[3] Ab attachment to surfaces falls into two categories: noncovalent and covalent.[4,5] We have previously shown that negatively charged polyelectrolyte capsules can be functionalized with antibodies using electrostatic interactions and be specifically targeted to cancer cells.[5] However, a critical factor governing targeted delivery is using a carrier that exhibits low levels of nonspecific binding to cells, making carriers assembled from low fouling, uncharged materials such as poly(ethylene glycol) (PEG) or poly(*N*-vinyl pyrrolidone) (PVPON) highly desirable. Electrostatic attachment is not effective for low fouling materials as they inhibit nonspecific protein binding. To functionalize such materials, covalent attachment of the Ab is required.

A variety of covalent coupling chemistries have been employed to attach antibodies to surfaces, principally carbodiimide, thiol/maleimide, and biotin/avidin coupling.[4] Click chemistry, using the Cu(I)-catalyzed azide–alkyne Huisgen cycloaddition,[6] is a versatile coupling strategy, as it proceeds readily in water, is rapid, has a high conversion efficiency, and has no unwanted side reactions. In this work, PVPON click capsules assembled by the layer-by-layer (LbL) technique were used as the carrier system; however, due to the general nature of click chemistry, this process could be applied to a wide range of delivery systems, including micelles and polymersomes.[1] The capsules were prepared by alternately layering PVPON functionalized with 1% alkyne moieties ($PVPON_{Alk}$) and poly(methacrylic acid) (PMA) on 585 nm-diameter silica templates, using hydrogen bonding to drive assembly. The alkyne groups on the PVPON were then cross-linked with a bifunctional azide linker. Upon core removal and treatment at pH 7 the capsules swell (to ~800 nm) and PMA is expelled from the capsule wall, leaving $PVPON_{Alk}$ capsules. Sufficient alkyne groups remained for additional functionalization steps.[7]

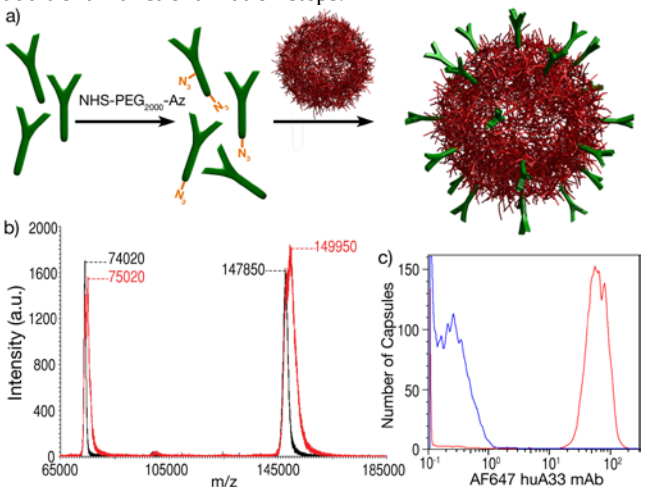

Figure 1. a) Scheme showing Ab azide functionalization with NHS-PEG_{2000}-Az linker, and subsequent capsule functionalization. b) MALDI spectrum of huA33 mAb (black) and azide-functionalized huA33 mAb (red). The secondary peak at ~74,000 is due to the $2H^+$ ion. c) Flow cytometry histogram of the fluorescence intensity of capsules (~3.8 μm diameter) incubated with AF647-labeled huA33 mAb_{Az} in the presence of chelated Cu(I) (red) and without Cu(I) catalyst (blue).

The Ab was functionalized with an azide by coupling a linear, bifunctional PEG with a succinimidyl ester group at one end (reactive towards lysine) and an azide at the other (NHS-PEG_{2000}-Az). The huA33 mAb contains 41 lysine residues, 26 of which are located in the F_c portion, so statistically the majority of functionalization will occur in the F_c portion.[3] Coupling was characterized using matrix-assisted laser desorption ionization (MALDI) spectroscopy (Figure 1b, S1). The primary peak in the MALDI spectrum shifted from 147 850 to 149 950 amu, corresponding to the molecular weight of one PEG_{Az} (2100 amu), indicating an average of one PEG_{Az} per Ab. However, the full width half maximum of the primary peak increased from 2000 to 5000 amu, indicating the actual degree of functionalization ranges from 0 to 2 PEG_{Az} per Ab. Click chemistry has been used previously to couple

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IgG molecules to the surface of superparamagnetic iron oxide particles;[8] however, we found that in the presence of the Cu(I) catalyst large Ab aggregates are formed (Figure S2), most probably due to complexation of Cu(I) with the carboxylic acid and amine groups present on the Ab. To overcome this, a chelator was used to complex the Cu(I) (Figure S3). No Ab aggregation was observed when Cu(I) was present in the chelated form.

Functionalization of $PVPON_{Alk}$ capsules with huA33 mAb_{Az} was qualitatively monitored with flow cytometry using Alexa Fluor 647 (AF647)-labeled Ab (Figure 1c). The average fluorescence intensity of capsules incubated in the presence of chelated Cu(I) was 67.8 a.u. (red spectra). In comparison, capsules incubated with Ab, but without Cu(I), exhibited an average intensity of 0.34 a.u. (blue spectra), clearly demonstrating the click attachment of Ab onto the capsule surface. From Ab adsorption experiments on ~800 nm diameter capsules, functionalization was determined to be 7 (±4) x 10^4 Ab per capsule (Figure S4). The activity of the immobilized Ab was confirmed by incubating the capsules with the AF488-labeled A33 antigen, which binds specifically to huA33 mAb. HuA33-functionalized capsules showed a high fluorescent signal (21.5 a.u.) compared to capsules prepared without Cu(I) (0.35 a.u.) and IgG-functionalized capsules (0.28 a.u.).

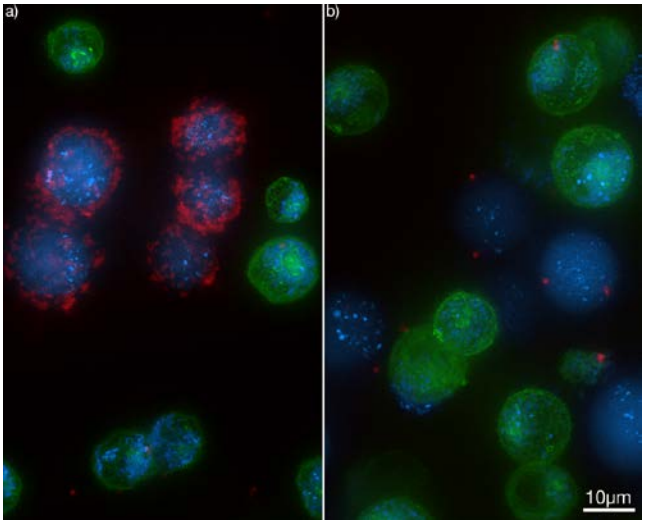

Figure 2. Fluorescence microscopy images of LIM2405+ cells (blue) and LIM2405– cells (green) incubated with a) huA33 mAb- and b) IgG-functionalized capsules (red).

The targeting specificity of the Ab-functionalized capsules was demonstrated using two stably transfected clones derived from the A33 antigen negative human colorectal cancer cell line, LIM2405. The first clone (LIM2405+), expressing human A33 antigen cDNA, was fluorescently labeled with CellTracker CMFDA and the second clone (LIM2405–), harboring the empty expression vector, was fluorescently labeled with LavaCell. A 50:50 suspension of the two clones was incubated at 4 °C for 1 h with a capsule to total cell ratio of 50:1 and analyzed using deconvolution fluorescence microscopy. Strikingly, while the LIM2405+ cells bound a large number of huA33 mAb-functionalized capsules, minimal binding of capsules to LIM2405– cells was observed (Figure 2a). Capsules functionalized in a similar way with IgG showed negligible nonspecific binding to both cell types (Figure 2b).

These results were confirmed with flow cytometry experiments, which showed that over 90% of LIM2405+ cells were associated with huA33 mAb-functionalized capsules, while less than 5% of the LIM2405– cells were associated with capsules (Figure 3a-c). The IgG-functionalized capsules showed limited nonspecific binding. Importantly, highly specific targeting was observed even when the LIM2405+ cells were present at less than 0.1% of the total cell population and the capsule to total cell ratio was 0.1:1 (Figure 3d). Under these conditions more than 50% of the LIM2405+ cells were associated with huA33 mAb capsules and nonspecific binding was negligible (<0.5%).

In conclusion, we have demonstrated the efficient click functionalization of nanoengineered capsules in the presence of a Cu(I) chelator to prevent aggregation of the Ab in the presence of Cu(I). These functionalized capsules show highly specific binding to cancer cells expressing the target antigen and minimal nonspecific binding, even when the capsule:target cell ratio is low. We expect this Ab functionalization technique to find a wide range of applications within the fields of bioconjugation, drug delivery, cellular imaging and sensing.

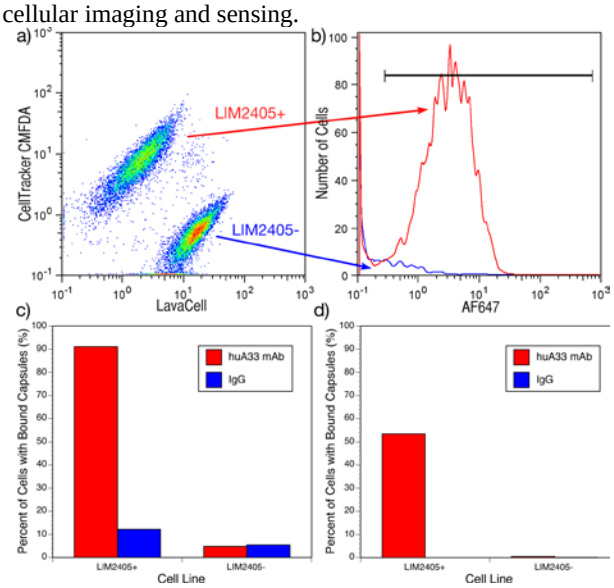

Figure 3. Flow cytometry analysis of capsules binding to mixed cell populations. a) Bivariant plot identifying LIM2405+ and LIM2405- cells. b) Histogram showing huA33 mAb-functionalized capsule binding to a 50:50 mixture of LIM2405+ (red) and LIM2405– (blue) cells incubated with a 50:1 capsule:cell ratio. Comparison of huA33 mAb and IgG-functionalized capsules incubated at c) 100:1 and d) 0.1:1 capsule:cell ratio with c) 50:50 and d) 0.1:99.9 ratio of LIM2405+:LIM2405– cells.

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Supporting Information Available: Experimental details, MALDI spectra, huA33 aggregation in the presence of Cu(I), and

quantification of Ab binding are available free of charge at http://pubs.acs.org.

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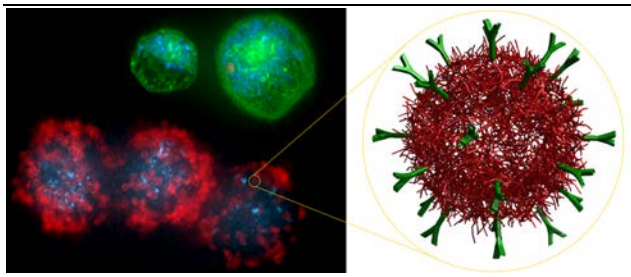

Targeted delivery of drugs to specific cells allows a high therapeutic dose to be delivered where the drug is required with minimal harmful side effects. Combining targeting molecules with nanoengineered drug carriers, such as polymer capsules, micelles and polymersomes, has significant potential to improve the therapeutic delivery of a range of drugs. We present a general approach to functionalize low fouling, nanoengineered polymer capsules with antibodies (Ab) using click chemistry. We demonstrate that Ab-functionalized capsules specifically bind to colorectal cancer cells, even when the target cells are less than 0.1% of the total cell population. This precise targeting offers promise for drug delivery applications.