J|A|C|S COMMUNICATIONS

Published on Web 10/26/2010

Targeting of Cancer Cells Using Click-Functionalized Polymer Capsules

Marloes M. J. Kamphuis,[†] Angus P. R. Johnston,[†] Georgina K. Such,[†] Henk H. Dam,[†] Richard A. Evans,[‡] Andrew M. Scott,[§] Edouard C. Nice,[||] Joan K. Heath,[||] and Frank Caruso*,[†]

Centre for Nanoscience and Nanotechnology, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria 3010, Australia, CSIRO Molecular and Health Technologies, Bayview Avenue, Clayton, Victoria 3168, Australia, Ludwig Institute for Cancer Research, Austin Hospital, Victoria 3084, Australia, and Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

Received July 20, 2010; E-mail: fcaruso@unimelb.edu.au

Abstract: Targeted delivery of drugs to specific cells allows a high therapeutic dose to be delivered to the target site 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 and index of a range of drugs. We present a general approach for functionalization of low-fouling, nanoengineered polymer capsules with antibodies using click chemistry. We demonstrate that antibody (Ab)-functionalized capsules specifically bind to colorectal cancer cells even when the target cells constitute less than 0.1% of the total cell population. This precise targeting offers promise for drug delivery applications.

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 should 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 using click chemistry. We demonstrate that antibody (Ab)-functionalized capsules bind specifically to cancer cells expressing the complementary antigen in vitro. This precise targeting occurs even when the target cells constitute 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 because of 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 specifically targeted to cancer cells.[5] However, a critical factor governing targeted delivery is the use of 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*-vinylpyrrolidone) (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, principally carbodiimide, thiol/maleimide, and biotin/avidin coupling, have been employed to attach antibodies to surfaces.[4] Click chemistry, which uses the Cu(I)-catalyzed azide−alkyne Huisgen cycloaddition,[6] is a versatile coupling strategy, as it proceeds readily in water, is rapid, and has a high conversion efficiency and 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, because of 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 the 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 swelled (to ~800 nm) and PMA was expelled from the capsule wall, leaving $PVPON_{Alk}$ capsules. Sufficient alkyne groups remained for additional functionalization steps.[7]

The Ab was functionalized with an azide by coupling a linear, bifunctional PEG with a succinimidyl ester group at one end (reactive toward 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 should occur in the F_c portion.[3] The coupling was characterized using matrix-assisted laser desorption ionization (MALDI) spectroscopy (Figure 1b and Figure S1 in the Supporting Information). The primary peak in the MALDI spectrum shifted from 147 850 to 149 950 amu; the mass difference corresponds to the molecular weight of one PEG_{Az} (2100 amu), indicating an average of one PEG_{Az} per Ab. However, the full width at half-maximum of the primary peak increased from 2000 to 5000 amu, indicating that the actual degree of functionalization ranged from 0 to 2 PEG_{Az} per Ab. Click chemistry has been used previously to couple IgG molecules to the surface of superparamagnetic iron oxide particles;[8] however, we found that large Ab aggregates formed in the presence of the Cu(I) catalyst (Figure S2), most probably as a result of 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 azide-functionalized huA33 mAb (huA33 mAb_{Az}) was qualitatively monitored with flow cytometry (Figure 1c). The average fluorescence intensity of capsules incubated in the presence of huA33 mAb_{Az} and chelated Cu(I) was 67.8 au (red spectrum). In comparison, capsules incubated

[†] The University of Melbourne.
[‡] CSIRO Molecular and Health Technologies.
[§] Ludwig—Austin Branch.
[||] Ludwig—Parkville Branch.

 © 2010 American Chemical Society

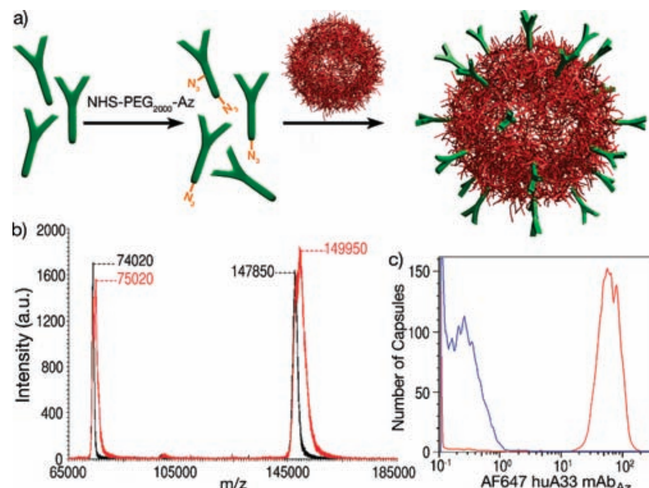

Figure 1. (a) Scheme showing Ab azide functionalization with the NHS−PEG_{2000}−Az linker and subsequent capsule functionalization. (b) MALDI spectrum of huA33 mAb (black) and Az-functionalized huA33 mAb_{Az} (red). The secondary peak at m/z 74 020 is due to the $[M + 2H]^{2+}$ ion. (c) Flow cytometry histogram of the fluorescence intensity of capsules (~3.8 μm diameter) incubated with Alexa Fluor 647 (AF647)-labeled huA33 mAb_{Az} in the presence of chelated Cu(I) (red) and without Cu(I) catalyst (blue).

with huA33 mAb_{Az} but without Cu(I) exhibited an average intensity of 0.34 au (blue spectrum), clearly demonstrating the click attachment of Ab onto the capsule surface. From Ab adsorption experiments on ~800 nm diameter capsules, the degree of functionalization was determined to be $(7 \pm 4) \times 10^4$ 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 stronger fluorescence signal (21.5 au) than capsules prepared without Cu(I) (0.35 au) or IgG-functionalized capsules (0.28 au).

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 complementary DNA, 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/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 the 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/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

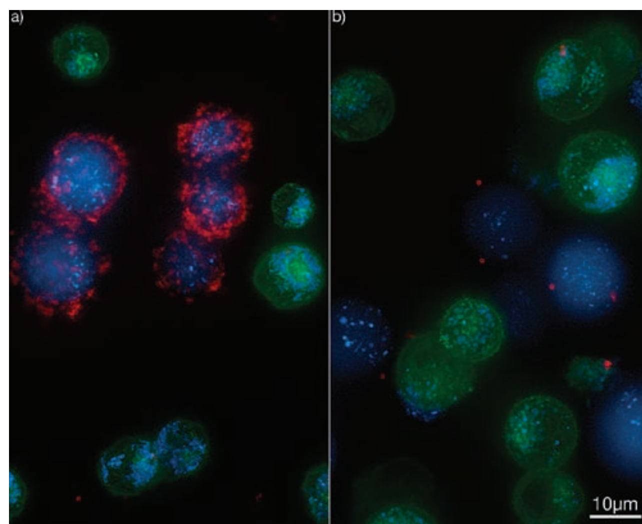

Figure 2. Fluorescence microscopy images of LIM2405+ cells (blue) and LIM2405− cells (green) incubated with (a) huA33 mAb- or (b) IgG-functionalized capsules (red). Capsule/cell ratio of 50:1. Scale bar corresponds to both images.

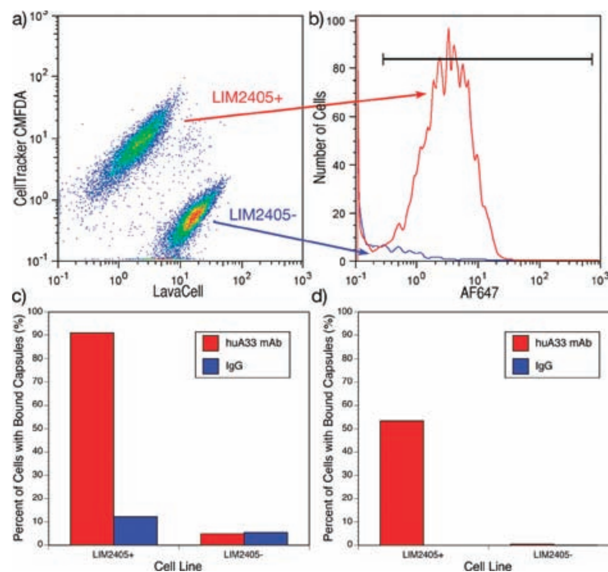

Figure 3. Flow cytometry analysis of binding of capsules to mixed cell populations. (a) Bivariant plot identifying LIM2405+ and LIM2405− cells. (b) Histogram showing binding of huA33 mAb-functionalized capsules to a 50:50 mixture of LIM2405+ (red) and LIM2405− (blue) cells incubated with a capsule/cell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

a Cu(I) chelator to prevent aggregation of the antibodies in the presence of Cu(I). These functionalized capsules showed highly specific binding to cancer cells expressing the target antigen and minimal nonspecific binding, even when the capsule/target cell ratio was 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.

Acknowledgment. We thank the ARC and NHMRC for funding under the Federation Fellowship (FF0776078), Discovery Project (DP0877360), and Program Grant (487922) schemes.

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

Ab binding, and complete ref 3. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) van Dongen, S. F. M.; de Hoog, H.-P. M.; Peters, R. J. R. W.; Nallani, M.; Nolte, R. J. M.; van Hest, J. C. M. *Chem. Rev.* **2009**, *109*, 6212–6274.
(2) Johnston, A. P. R.; Cortez, C.; Angelatos, A. S.; Caruso, F. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 203–209.
(3) Scott, A. M.; et al. *Clin. Cancer Res.* **2005**, *11*, 4810–4817.
(4) Shi, M.; Lu, J.; Shoichet, M. S. *J. Mater. Chem.* **2009**, *19*, 5485–5498.
(5) Cortez, C.; Tomaskovic-Crook, E.; Johnston, A. P. R.; Radt, B.; Cody, S. H.; Scott, A. M.; Nice, E. C.; Heath, J. K.; Caruso, F. *Adv. Mater.* **2006**, *18*, 1998–2003.
(6) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
(7) Kinnane, C. R.; Such, G. K.; Antequera-Garcia, G.; Yan, Y.; Dodds, S. J.; Liz-Marzan, L. M.; Caruso, F. *Biomacromolecules* **2009**, *10*, 2839–2846.
(8) Thorek, D. L. J.; Elias, D. R.; Tsourkas, A. *Mol. Imaging* **2009**, *8*, 221–229.

JA106405C