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Local Release of Highly Loaded Antibodies from Functionalized Nanoporous Support for Cancer Immunotherapy

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Recent advances with functionalized nanoporous supports provide an innovative approach for entrapping proteins and for their subsequent controlled release and delivery.[1–7] Functionalized mesoporous silica (FMS) can provide a confined and interactive nanoenvironment that increases protein activity and allows large amounts of protein loading compared to unfunctionalized mesoporous silica (UMS) or normal porous silica.[5–7] First, the proteins can be spontaneously entrapped in FMS with a rigid, uniform, open nanopore geometry of tens of nanometers via noncovalent interaction. Then, one can control the release of the entrapped proteins from FMS based on the function groups and pore sizes when the FMS-protein composites are dispersed in a fresh buffer solution in which a new thermodynamic balance can be reached. In this work, we found that antibodies can be spontaneously loaded in FMS with superhigh density (0.4−0.8 mg of antibody/mg of FMS) due to their comprehensive noncovalent interaction. We hypothesize that therapeutic antibodies entrapped in FMS can be gradually released locally *in vivo* under physiological conditions and that this will help develop innovative therapies for many diseases. We performed pilot tests to investigate the antitumor activity of a monoclonal antibody (mAb) to CTLA4,[8] an immunoregulatory molecule released from FMS at the tumor site. This strategy resulted in a much greater and extended inhibition of tumor growth than the antibody given systemically.

To ensure a large loading of mAb molecules (M.W. 150 kDa) in FMS, we prepared UMS with a pore size (diameter) as large as 30 nm, a surface area as great as 533 m^2/g, and an average bead size of 12−15 μm (Supporting Information).[9,10] A controlled hydration and condensation reaction was used to introduce functional groups into UMS.[9,10] Coverage of 2% (or 20%) HOOC-FMS, HO_3S-FMS or NH_2-FMS means 2% (or 20%) of the total available silanol groups (5×10^{18} silanol groups per square meter[9,10]) of UMS would be silanized with trimethoxysilane with the functional group HOOC, HO_3S, or NH_2.[1–7] Figure 1A shows the TEM image of 30 nm 20% HOOC-FMS. There is no significant difference between the TEM images of UMS and their corresponding FMS.[6] Unlike 3-nm and 10-nm mesoporous silica, the 30-nm mesoporous silica has a large degree of disorder,[11] but it still reveals a more or less uniform cage-like porous structure.[12]

FMS was incubated in the antibody solution, where the antibody would be spontaneously entrapped in FMS. We defined the protein amount (mg) of an antibody entrapped with 1 mg of FMS as the protein-loading density (P_{LD}). We first exploited the large loading density of FMS for entrapping rat and mouse IgGs and studying their releasing ability in a physiological buffer (Figure 1B and Supporting Information, Figure S1). IgGs were loaded in various FMSs. The resulting FMS-IgG composites were then transferred to fresh buffers and eluted multiple times to determine the release kinetics of the antibody from the particles. The protein contents of the supernatants in between each cycle of shaking−elution−centrifugation were measured. Although different, P_{LD} values of IgGs in various FMSs were all superhigh at the "0 elution" data point (0.4−0.8 mg of IgG/mg of FMS), which is much higher than previously reported for other proteins.[1–7] The subsequent controllable release of the IgG from FMS was carried out in pH 7.4, 10 mM sodium phosphate, 0.14 M NaCl (PBS), or a simulated body fluid that has ion concentrations nearly equal to those of human blood plasma (buffered at pH 7.4 with 50 mM Tris-HCl) (Figure 1B and Supporting Information, Figure S1). A decreasing P_{LD} was observed along the series of elutions. For both rat and mouse IgGs, the 20% HOOC-FMS and 2% HO_3S-FMS displayed faster releasing rates than other FMSs under the identical elution solutions. These results reflected the difference of the comprehensive noncovalent interaction of IgG with various FMSs, that is, the electrostatic, H-bond, hydrophilic, and hydrophobic interaction of the functional groups and spacers of FMS with the amino acid residues of protein molecules.[5]

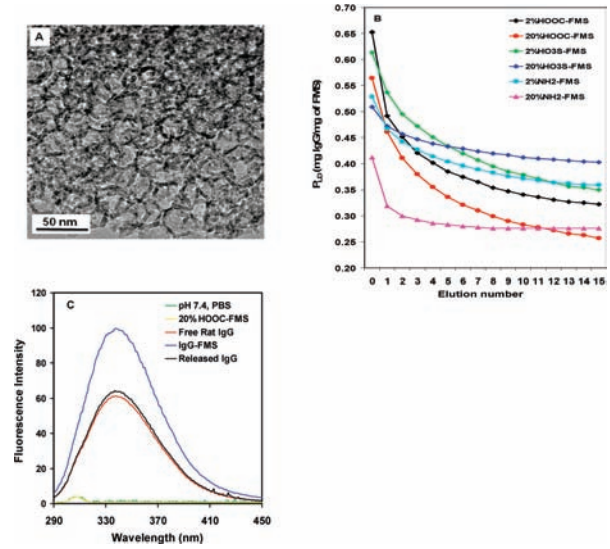

Figure 1. (A) TEM image of 30 nm 20% HOOC-FMS. (B) Rat IgG loading density in FMS and gradual release of the IgG from FMS in the simulated body fluid. (C) Fluorescence spectra of the free rat IgG, the FMS-IgG, and the released IgG from FMS. [IgG]: 0.03 mg/mL in pH 7.4, PBS. The excitation was at 278 nm.

Figure 1C shows fluorescence emission spectra of the free rat IgG, the entrapped IgG in FMS, and the released IgG from FMS. Fluorescence emission was monitored at the excitation wavelength of 278 nm, allowing excitation of both tyrosinyl and tryptophanyl residues.

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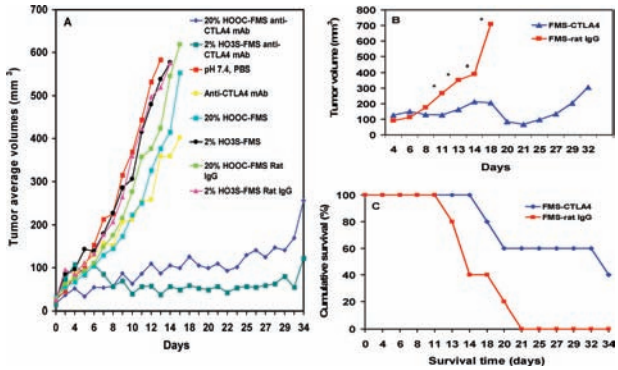

Figure 2. (A) Antitumor activity of FMS-anti-CTLA4 injected subcutaneously (s.c.) into small established, growing mouse melanomas (3 mice/group). 0.5 mg Anti-CTLA4 was used. Controls were the PBS buffer, anti-CTLA4, FMS (20% HOOC- and 2% HO_3S-), and FMS-Rat IgG. (B) Summary results of antitumor activity of 20% HOOC-FMS-anti-CTLA4 from a repeat experiment with 5 mice/group which had small SW1 tumors on both sides of the back, providing 10 tumor sites/group. Two tumors were completely regressed. *$p <$ 0.05. (C) Survival of mice in the repeat experiment.

Comparing the free IgG to FMS-IgG (Figure 1C), we observed no dramatic emission peak shift but increased emission intensity because of the interaction of IgG with FMS, which might result in less exposure of tyrosinyl and tryptophanyl residues to the aqueous environment. It is noteworthy that the released IgG displayed similar fluorescence spectra to that of the free IgG prior to the entrapment, indicating that the interaction of FMS with IgG did not induce a dramatic change on the IgG protein structure. Our preliminary result also shows that the *in vitro* released antibody from FMS still maintained its binding activity (Supporting Information, Table S1).

Monoclonal antibodies have been used to treat many medical conditions, including cancer.[13–15] For example, a systemic administration of an mAb to the immunoregulatory molecule CTLA4 has displayed antitumor activity by modifying the host response to tumors, both in mouse models and in human cancer patients.[16] It is important that a sufficient amount of the mAb is delivered to the tumor, as the tumor microenvironment is highly immunosuppressive because of its high concentration of tumor antigen, regulatory T lymphocytes, etc.[17] However, to deliver sufficient amounts of the anti-CTLA4 mAb to a tumor to be therapeutically effective, there is a risk of side effects from inducing autoimmunity to normal tissue antigens. For example, a profound antitumor activity was marred by toxicity in several renal carcinoma patients who had been injected systemically with anti-CTLA4 mAb.[18]

To test our strategy, we selected a rat IgG mAb to CTLA4 for entrapment into FMS particles.[8] The FMS-entrapped antibody was injected directly into established mouse melanomas derived from s.c. injection of cells from the SW1 clone. We compared the results to several controls, including intraperitoneally injected anti-CTLA4 mAb and intratumorally injected FMS particles as well as FMS particles containing rat IgG and PBS buffer. Mice were injected with 10^6 SW1 cells s.c. on the back. When the mice had tumors of ~3 mm mean diameter, we randomized them according to tumor size into different groups, each comprised of three mice. Figure 2A shows representative results from each treatment group. The results demonstrate that FMS-anti-CTLA4 inhibited tumor growth. We saw no evidence of toxicity from injecting FMS particles into tumors. In particular, the antitumor activity of FMS-Anti-CTLA4 (>50% tumor regression) was much more potent than that of anti-CTL4 alone (without FMS). We have repeated the experiment and observed similar results (Figure 2B and 2C). To confirm the local release, we measured the *in vivo* release of fluorescent dye-labeled IgG from FMS at the tumor site. The results demonstrate that FMS entrapping with IgG prolonged the antibody stay at the tumor site and thus facilitates sustained antibody release in tumors, offering an advantage over simply injecting antibodies into tumors (Supporting Information, Figure S2). Futher optimization of functionalization and pore sizes of FMS,[4,19] more extensive therapeutic and pathological experiments are ongoing, and the results will be reported elsewhere.

We conclude that immunoglobulins can be loaded in FMS particles with superhigh density to provide long-lasting local release, and our preliminary data indicate that FMS-entrapped anti-CTLA4 IgG mAb induces a much greater and extended therapeutic response than the same amount given systemically. Our results have also demonstrated that the rate and durability of the mAb release from FMS particles can be fine-tuned by changing the functional group types and coverages of FMS (Figure 1B and Supporting Information, Figure S1). We expect that a similar approach of local release can be applied to other mAbs as well as other immunologically active proteins, delivered alone or in combination, and that a long-lasting local release will cause more effective tumor destruction with less dose amounts, longer dose intervals, and thus fewer side effects than systemic administration.

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Supporting Information Available: Experimental section and additional experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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