Cancer Therapy

Deutsche Ausgabe: DOI: 10.1002/ange.201506179
Internationale Ausgabe: DOI: 10.1002/anie.201506179

Stimulation of In Vivo Antitumor Immunity with Hollow Mesoporous Silica Nanospheres

Xiupeng Wang[+], Xia Li[+], Atsuo Ito, Yohei Watanabe, Yu Sogo, Noriko M. Tsuji, and Tadao Ohno*

Abstract: *The use of appropriate adjuvants that support the generation of robust and long-lasting antitumor immune responses is crucial for tumor immunotherapy owing to the immunosuppressive environment of the growing tumor. However, the most commonly used adjuvant, aluminum hydroxide, is ineffective for generating such immune responses and therefore not suitable for cancer immunotherapy. It is now shown that plain hollow mesoporous silica nanospheres markedly improve the antitumor immunity, the Th1 and Th2 immunity, and the CD4$^+$ and CD8$^+$ effector memory T cell population in bone marrow in vivo and may thus be used as immunoadjuvants to treat cancer in humans.*

Cancer is still a leading cause of death worldwide, despite the tremendous efforts devoted to cancer research. Most deaths of cancer patients are due to metastasis,[1] because traditional tumor therapies have limited effects in these patients. Cancer immunotherapy, a treatment that harnesses and enhances the innate power of the immune system to fight cancer, is particularly efficient for the specific treatment of recurrent or metastasized cancer without damaging normal tissues.[2]

The use of appropriate adjuvants for generating a robust and long-lasting adaptive antitumor response is crucial in cancer immunotherapy.[3] Generally, by the time a tumor has become detectable, it has developed an immune inhibition that limits or down-regulates antitumor immune responses. As the most commonly used adjuvant, aluminum hydroxide (alum), fails to generate robust and long-lasting antitumor immune responses, the development of new adjuvants suitable for cancer immunotherapy is an important challenge.

Mesoporous silica (MS), which benefits from good biocompatibility,[4] large surface areas, a uniform pore structure, and easily tunable particle sizes, morphology, surface properties, has been intensively studied for biomedical applications, including imaging,[5] drug delivery,[6] and cancer diagnosis and therapy.[7] Although several studies have indicated that MS was a potential immunoadjuvant owing to its high surface area and effective molecule adsorption, the effect of plain MS on the antitumor immunity and immune memory is still not fully understood.[8]

Herein, hollow mesoporous silica (HMS) nanospheres were synthesized and used as cancer immunoadjuvants. We investigated whether the HMS nanospheres 1) generate systemic antitumor immunity, 2) promote immune memory, and 3) stimulate Th1 antitumor immunity in vivo.

Monodisperse HMS nanospheres were synthesized by a surfactant-assembly sol–gel process in a Stöber solution containing hexadecyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), ammonia, and ethanol according to a modified version of a previously published procedure.[9] The HMS nanospheres had a smooth surface, uniform diameters of approximately 200 nm, and a shell thickness of about 30–40 nm (Figure 1 A–C). The shell of the HMS nanospheres contained mesopores with diameters of approximately 3–6 nm (Figure 1 B). The type IV isotherm curves with a hysteresis loop in the P/P_0 range of 0.5–1.0 (Figure 1 D) also indicated that the HMS nanospheres possessed a mesoporous structure. The BET surface area of the HMS nanospheres was 1154 m^2g^{-1}. The pore size distribution of the HMS nanospheres also revealed mesopores with diameters of about 3–6 nm (Figure 1 E) and is thus consistent with the TEM results. The HMS nanospheres consist of amorphous silica as shown by a broad peak at approximately 15–30° in the XRD pattern (Figure 1 F) and Si–O absorption bands at 1040, 800, and 470 cm^{-1} in the FTIR spectrum (Figure 1 G). The HMS nanospheres were negatively charged in calcium- and magnesium-free phosphate-buffered saline with zeta potentials of −25 mV (Figure 1 H).

When macrophage-like cells are treated with fluorescent conjugates of lipopolysaccharides (F-LPS) for four hours, they take up only a small fraction of the model biomolecules (Figure 2 A). When F-LPS was mixed with alum, the cellular uptake of F-LPS was slightly improved. In contrast, when F-LPS was mixed with HMS, the cellular uptake of F-LPS was even better than for either F-LPS alone or the F-LPS/alum mixture (Figure 2 A). Quantitative evaluation of the cellular

[*] Dr. X. Wang,[+] Dr. X. Li,[+] Dr. A. Ito, Dr. Y. Sogo
Health Research Institute
Department of Life Science and Biotechnology
National Institute of Advanced Industrial Science and Technology (AIST)
Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566 (Japan)
E-mail: xp-wang@aist.go.jp
Y. Watanabe, Dr. N. M. Tsuji
Biomedical Research Institute
Department of Life Science and Biotechnology
National Institute of Advanced Industrial Science and Technology (AIST)
Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566 (Japan)
Dr. T. Ohno
School of Life Dentistry at Tokyo
The Nippon Dental University
Fujimi, Chiyoda-ku Tokyo 102-0071 (Japan)
[+] These authors contributed equally to this work.

Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under http://dx.doi.org/10.1002/anie.201506179.

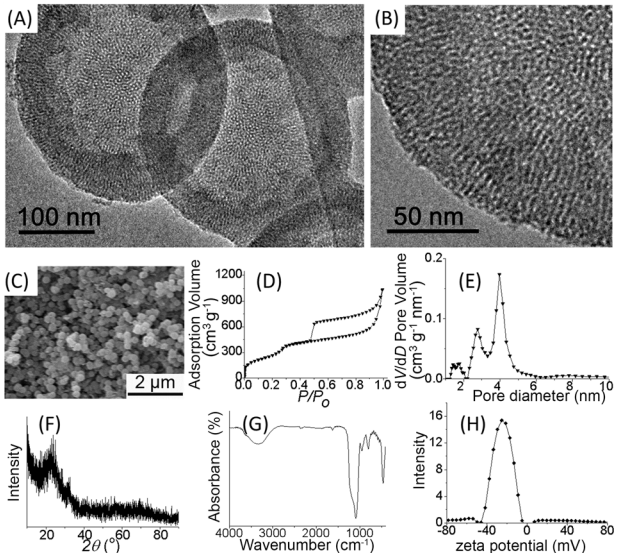

Figure 1. Physicochemical characterization of the HMS nanospheres. A, B) TEM images with different magnification. C) SEM image. D) N_2 adsorption–desorption isotherm. E) Pore size distribution. F) XRD pattern. G) FTIR spectrum. H) Zeta potential distribution.

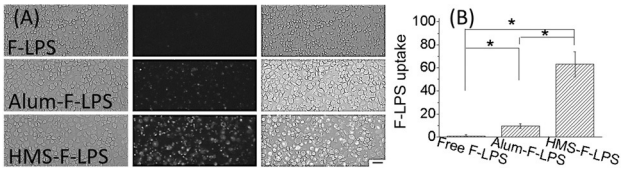

Figure 2. The HMS nanospheres significantly promote F-LPS uptake by macrophage-like cells after 4 h in culture medium, compared with alum-F-LPS and free F-LPS. A) Representative images of the F-LPS uptake: bright-field cell images (left), fluorescence images (middle), and merged images (right). Scale bar: 10 μm. B) Quantitative F-LPS uptake ($n = 6$, * $p < 0.05$).

uptake of F-LPS using a fluorescence microplate reader showed that HMS mixed with F-LPS improved the cellular uptake of F-LPS by factors of 63.1 and 6.5 compared with that of F-LPS alone and F-LPS mixed with alum, respectively (Figure 2B).

MS materials are able to deliver molecules efficiently to immune cells, control their release, and induce an immune response, while protecting the delivered immune-stimulating molecules from degradation under physiological conditions. MS materials have recently been widely employed as carriers for the controlled delivery of therapeutic substances, such as drugs, proteins, and other biogenic molecules.[6,10] Previous studies have shown that molecular drugs can be entrapped within the mesopores by an impregnation process and released by a diffusion-controlled mechanism. The interactions between the MS and biomolecules include hydrogen bonding, electrostatic interactions, and hydrophobic interactions.[11] Furthermore, owing to the large cavity inside each HMS nanosphere, about 3–15 times higher loading capacities of small anticancer drug molecules were achieved compared with MS without large cavities.[12] Herein, the HMS nanospheres exhibited strong affinities to various biomolecules. First, the high adsorption affinity of a model protein, ferritin, towards the HMS nanospheres was clearly visualized. The original HMS nanospheres showed particle edges and mesopores with diameters of 3–6 nm when analyzed by transmission electron microscopy (Figure 1; see also the Supporting Information, Figure S1). After adsorption, however, the channels and/or surfaces of the HMS nanospheres were filled with/covered by ferritin molecules (Figure S1). Furthermore, tumor antigen and fluorescein conjugates of ovalbumin (F-OVA) were used to further confirm the strong affinity of the HMS nanospheres towards biomolecules. For instance, 68% of the tumor antigens and 28% of F-OVA in the original solutions had been adsorbed onto the HMS nanospheres after mixing the tumor antigen or F-OVA with HMS at 4 °C for 24 h (Figure S2A, B). HMS nanospheres that had adsorbed the tumor antigen or F-OVA only slowly released these compounds, as only 33% of the adsorbed tumor antigens and 21% of the adsorbed F-OVA had been released after seven days (Figure S2C, D).

Then, the simultaneous internalization of rhodamine B grafted HMS nanospheres and F-LPS into macrophage-like cells was confirmed by confocal microscopy images recorded after four hours of incubation. Both the rhodamine B grafted HMS nanospheres and F-LPS were taken up to a significant extent by the macrophage-like cells in vitro. Moreover, the colocalization of the rhodamine B grafted HMS nanospheres and F-LPS indicated that the F-LPS was still efficiently adsorbed onto the HMS nanospheres when they were engulfed by macrophage-like cells (Figure S3). The high adsorption affinity of the HMS nanospheres towards biomolecules explains the significant increase in cellular uptake of biomolecules.

Plain HMS nanospheres greatly inhibited tumor challenge and re-challenge (Figures 3 and 4), indicating that they can be used as cancer immunoadjuvants. Using Lewis lung carcinoma (LLC) cell fragments as a tumor antigen, C57BL/6 mice were first immunized with HMS nanospheres or without the adjuvant, and then challenged with live LLC cells. Mice immunized with plain HMS nanospheres showed significant

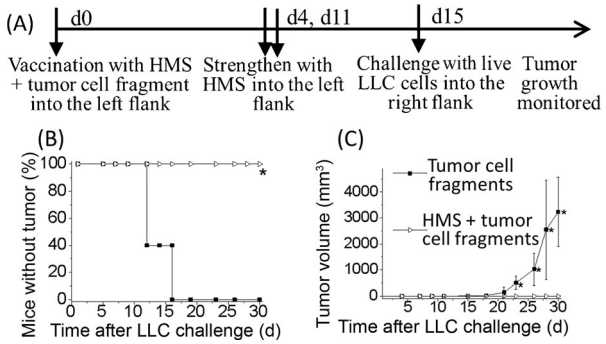

Figure 3. HMS nanospheres mixed with tumor cell fragments exhibit significant antitumor immunity in a prior immunization model. A) Experimental procedure. B) Percentage of mice without tumor. C) Tumor volume after immunization and challenge with live LLC cells ($n = 5$, * $p < 0.05$).

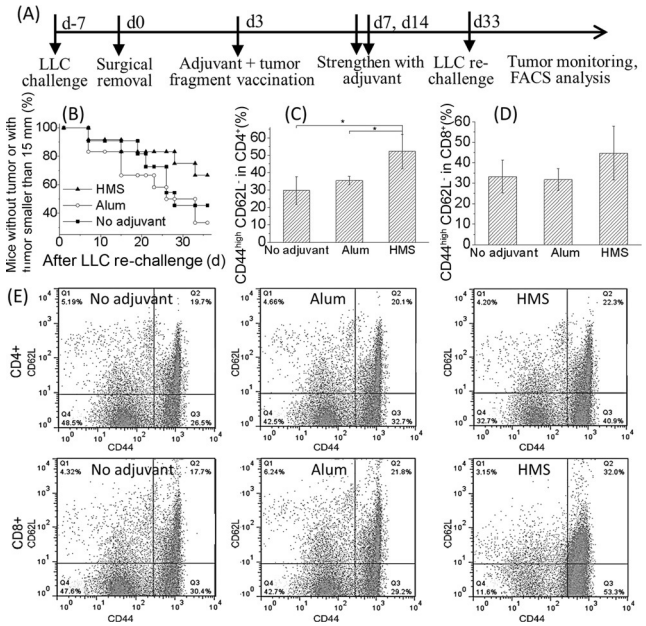

Figure 4. HMS nanospheres mixed with autologous tumor fragments exhibit significant antitumor immunity in a re-challenge model. A) Experimental procedure. B) Percentage of mice without tumor or with tumors smaller than 15 mm after LLC re-challenge ($n = 11$–12, * $p < 0.05$). C–E) Adjuvant effects on memory T cells ($n = 3$, * $p < 0.05$). $CD4^+$ (C, E) and $CD8^+$ (D, E) memory T cell population in the bone marrow of mice two months after vaccination. E) Representative results. C, D) Summary of results shown in (E).

inhibition of the LLC tumor challenge compared with those immunized without the adjuvant (Figure 3). In all of the mice immunized with the plain HMS nanospheres, tumor growth was inhibited for at least 30 days after the LLC challenge. In contrast, all mice immunized without the adjuvant had developed tumors with volumes of $3236 \pm 1329\ mm^3$ 30 days after the LLC challenge. The adjuvant effect of the plain HMS nanospheres was further confirmed by a tumor re-challenge model (Figure 4A, B). Using autologous LLC tumor fragments as a tumor antigen, the C57BL/6 mice were first immunized with HMS nanospheres, alum, or without an adjuvant, and then re-challenged with live LLC cells. Mice immunized with the plain HMS nanospheres showed greatly inhibited tumor growth compared with those immunized with alum or without an adjuvant.

The immune-stimulating and -directing properties of MS may explain the high antitumor immunity conveyed by the plain HMS nanospheres. MS particles were reported to increase the number of CD86 cells in human-monocyte-derived dendritic cells, tune the development of naive T cells through dendritic cell signaling, promote cytokine secretion in vitro,[8d,e] and increase both the IgG2a and IgG1 isotypes in vivo.[8c,13] Using the purified PCV2 GST-ORF2-E protein as an antigen, the HMS particles have already been shown to promote $CD4^+$ and $CD8^+$ splenocyte proliferation in vivo.[8a]

Herein, we show that the plain HMS nanospheres markedly improve the population of $CD4^+$ and $CD8^+$ effector memory T cells in the bone marrow of mice two months after the vaccination compared with those immunized by alum or without an adjuvant (Figure 4C–E). The $CD4^+$ effector memory T cell populations in the bone marrow of mice treated with the plain HMS nanospheres, alum, or without an adjuvant were $(52.3 \pm 9.8)\%$, $(35.5 \pm 2.5)\%$, and $(29.7 \pm 8.1)\%$, respectively (Figure 4C). The corresponding values for the $CD8^+$ effector memory T cell populations are $(44.6 \pm 13.2)\%$, $(31.7 \pm 5.4)\%$, and $(33.2 \pm 8.0)\%$, respectively (Figure 4D). Generally, vaccines protect the body against infections because of immune memory. The immune memory is a key feature of the adaptive immune system that is maintained in the body by the B and T lymphocytes for years.[14] Therefore, the immunological memory that allows the adaptive immune system to rapidly clear previously encountered cancer antigens is strongly required for cancer immunotherapy.

Moreover, the plain HMS nanospheres significantly improved the secretion of both chicken egg ovalbumin (OVA) specific Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-10) cytokines of lymphocytes ex vivo compared with the immunization with alum or without adjuvant (Figure 5A–D). The plain HMS nanospheres achieved maximum OVA-specific IFN-γ, IL-2, IL-4, and IL-10 secretion values of (575.7 ± 240.0), (34.3 ± 7.9), (10.1 ± 17.8), and $(388.7 \pm 271.7)\ pg\,mL^{-1}$, respectively. Th1 immunity is very important for tumor immunotherapy as patients with advanced cancer often have impaired-cell-mediated immunity associated with a switch from Th1 to Th2.[15] Th1 cells, which are characterized

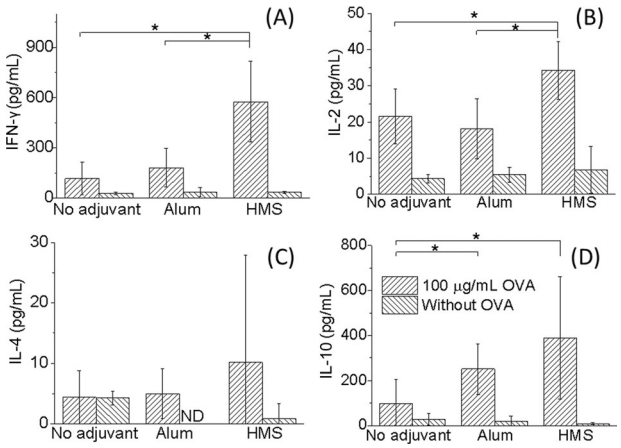

Figure 5. HMS nanospheres induce the secretion of OVA-specific cytokines highly related to antitumor immunity by the lymphocytes after 3 days in culture medium ex vivo ($n = 3$*3, ND = not detected, * $p < 0.05$).

by the secretion of IL-2, IL-12, IFN-γ, and TNF-α, exert potent antitumor effects by activating antigen-presenting cells, $CD8^+$ cytotoxic T lymphocytes, and natural killer cells. The plain HMS nanospheres are a more promising adjuvant than alum as they stimulate both Th1 and Th2 immunity. In contrast, the alum adjuvant induces a strong Th2 immunity, but is rather ineffective for Th1 immunity.[16]

An ideal adjuvant should act as both an antigen delivery vehicle and an immune potentiator.[17] Most of the traditional adjuvants are able to slowly release antigens by forming an

antigen depot at the injection site, thereby sustaining the antigen exposure of the immune system for a longer time and eliciting a stronger immune response. However, a major obstacle to the development of cancer immunotherapy is the immunosuppressive environment of the growing tumor. Therefore, the use of particulate adjuvants loaded with pathogen-associated molecular patterns (PAMPs) or cytokines for cancer immunotherapy has recently come into focus.[18] The particulate adjuvants mostly act as carriers of PAMPs or cytokines. Thus the efficacy of plain particulate adjuvants in enhancing and directing antitumor immune responses had previously not been fully recognized. Herein, the HMS nanospheres not only greatly improved the cellular uptake of a protein (Figure 2) and prolonged the release of the tumor antigen (Figure S2), but most importantly, they can effectively stimulate an immune response in the absence of immune potentiators. Overall, simple PAMP-free HMS nanospheres may be used as immunoadjuvants for the treatment of human cancers, as they have a simple composition, are inexpensive to manufacture, safe to administer, easily taken up by immune cells, and effectively elicit the desired antitumor immune response, immune memory, and Th1 and Th2 immunity in vivo.

Mesoporous silica has been extensively used in the biomedical field over the past two decades and shown excellent biocompatibility with many biological systems,[4–7] which paves the way for its clinical application. Silica is an endogenous substance of the human body that is particularly abundant in supporting tissues.[4c] Silica is often used as an excipient in pills and frequently taken as a dietary supplement for nourishing hair, skin, and nails.[4c] Mesoporous silica is a biodegradable material and can be degraded by simulated body fluids within 15 days in vitro.[4a] In vivo studies have shown that 94.4 % of the mesoporous silica administered by intraperitoneal injection (50 mg kg^{-1}) was excreted in the urine and feces within four days.[4b] Recently, silica nanoparticles in the form of Cornell dots, which assist both the diagnosis and targeted treatment of cancer cells, were approved by the U.S. Food and Drug Administration (FDA) for human clinical trials. The results showed no toxic or adverse events attributable to the silica particles, highlighting the great potential of silica nanoparticles in clinical applications.[19]

In conclusion, monodisperse HMS nanospheres with diameters of 200 nm and a shell thickness of 30–40 nm have been synthesized and successfully used as cancer immunoadjuvants. Interestingly, vaccination with plain HMS/autologous tumor fragments greatly inhibited in vivo tumor growth compared with vaccination with alum or without an adjuvant after re-challenge with LLC cells. Flow cytometry analysis showed that the HMS nanospheres markedly increased the population of $CD4^+$ and $CD8^+$ effector memory T cells in the bone marrow of mice two months after the vaccination. Furthermore, HMS nanospheres loaded with OVA significantly improved the secretion of the OVA-specific cytokines Th1 and Th2. The HMS nanospheres therefore are more efficient adjuvants than alum in cancer immunotherapy.

Acknowledgements

We thank Kazuko Yoshiyuki, AIST for technical assistance with the ELISA analysis and animal experiments. This study was supported in part by KAKENHI (Grant-in-Aid for Young Scientists B, 26750162 and 23700567).

Keywords: adjuvants · antitumor immunity · cancer · immune memory · silica nanospheres

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 1899–1903
Angew. Chem. **2016**, *128*, 1931–1935

[1] C. L. Chaffer, R. A. Weinberg, *Science* **2011**, *331*, 1559–1564.
[2] a) J. N. Blattman, P. D. Greenberg, *Science* **2004**, *305*, 200–205; b) S. A. Rosenberg, J. C. Yang, N. P. Restifo, *Nat. Med.* **2004**, *10*, 909–915; c) S. A. Rosenberg, *Nature* **2001**, *411*, 380–384.
[3] a) R. F. Service, *Science* **2005**, *310*, 1132–1134; b) S. T. Reddy, A. J. van der Vlies, E. Simeoni, V. Angeli, G. J. Randolph, C. P. O'Neill, L. K. Lee, M. A. Swartz, J. A. Hubbell, *Nat. Biotechnol.* **2007**, *25*, 1159–1164; c) A. Wack, R. Rappuoli, *Curr. Opin. Immunol.* **2005**, *17*, 411–418; d) R. T. Kenney, R. Edelman, *Expert Rev. Vaccines* **2003**, *2*, 167–188; e) S. G. Reed, S. Bertholet, R. N. Coler, M. Friede, *Trends Immunol.* **2009**, *30*, 23–32.
[4] a) Q. J. He, J. L. Shi, M. Zhu, Y. Chen, F. Chen, *Microporous Mesoporous Mater.* **2010**, *131*, 314–320; b) J. Lu, M. Liong, Z. X. Li, J. I. Zink, F. Tamanoi, *Small* **2010**, *6*, 1794–1805; c) J. M. Rosenholm, V. Mamaeva, C. Sahlgren, M. Linden, *Nanomedicine* **2012**, *7*, 111–120.
[5] W. P. Fan, B. Shen, W. B. Bu, F. Chen, Q. J. He, K. L. Zhao, S. J. Zhang, L. P. Zhou, W. J. Peng, Q. F. Xiao, D. L. Ni, J. N. Liu, J. L. Shi, *Biomaterials* **2014**, *35*, 8992–9002.
[6] a) M. Ma, Y. Huang, H. R. Chen, X. Q. Jia, S. G. Wang, Z. Z. Wang, J. L. Shi, *Biomaterials* **2015**, *37*, 447–455; b) D. C. Niu, Z. J. Liu, Y. S. Li, X. F. Luo, J. Y. Zhang, J. P. Gong, J. L. Shi, *Adv. Mater.* **2014**, *26*, 4947–4953; c) X. Li, L. X. Zhang, X. P. Dong, J. Liang, J. L. Shi, *Microporous Mesoporous Mater.* **2007**, *102*, 151–158.
[7] J. L. Shi, Y. Chen, H. R. Chen, *Int. J. Inorg. Mater.* **2013**, *28*, 1–11.
[8] a) H. C. Guo, X. M. Feng, S. Q. Sun, Y. Q. Wei, D. H. Sun, X. T. Liu, Z. X. Liu, J. X. Luo, H. Yin, *Virol. J.* **2012**, *9*, 108; b) D. Mahony, A. S. Cavallaro, F. Stahr, T. J. Mahony, S. Z. Qiao, N. Mitter, *Small* **2013**, *9*, 3138–3146; c) L. P. Mercuri, L. V. Carvalho, F. A. Lima, C. Quayle, M. C. A. Fantini, G. S. Tanaka, W. H. Cabrera, M. F. D. Furtado, D. V. Tambourgi, J. D. R. Matos, M. Jaroniec, O. A. Sant'Anna, *Small* **2006**, *2*, 254–256; d) H. Vallhov, N. Kupferschmidt, S. Gabrielsson, S. Paulie, M. Stromme, A. E. Garcia-Bennett, A. Scheynius, *Small* **2012**, *8*, 2116–2124; e) H. Vallhov, S. Gabrielsson, M. Stromme, A. Scheynius, A. E. Garcia-Bennett, *Nano Lett.* **2007**, *7*, 3576–3582.
[9] Z. G. Teng, X. D. Su, Y. Y. Zheng, J. Sun, G. T. Chen, C. C. Tian, J. D. Wang, H. Li, Y. N. Zhao, G. M. Lu, *Chem. Mater.* **2013**, *25*, 98–105.
[10] a) X. P. Wang, X. Li, A. Ito, Y. Sogo, *Acta Biomater.* **2011**, *7*, 3638–3644; b) X. P. Wang, X. Li, K. Onuma, A. Ito, Y. Sogo, K. Kosuge, A. Oyane, *J. Mater. Chem.* **2010**, *20*, 6437–6445.
[11] C. Mathé, S. Devineau, J. C. Aude, G. Lagniel, S. Chédin, V. Legros, M. H. Mathon, J. P. Renault, S. Pin, Y. Boulard, J. Labarre, *Plos One* **2013**, *8*, e81346.
[12] a) L. L. Li, F. Q. Tang, H. Y. Liu, T. L. Liu, N. J. Hao, D. Chen, X. Teng, J. Q. He, *ACS Nano* **2010**, *4*, 6874–6882; b) F. Chen, H. Hong, S. X. Shi, S. Goel, H. F. Valdovinos, R. Hernandez, C. P. Theuer, T. E. Barnhart, W. B. Cai, *Sci. Rep.* **2014**, *4*, 5080.

[13] L. V. Carvalho, R. D. Ruiz, K. Scaramuzzi, E. B. Marengo, J. R. Matos, D. V. Tambourgi, M. C. A. Fantini, O. A. Sant'Anna, *Vaccine* **2010**, *28*, 7829 – 7836.
[14] a) M. B. Fuertes, A. K. Kacha, J. Kline, S. R. Woo, D. M. Kranz, K. M. Murphy, T. F. Gajewski, *J. Exp. Med.* **2011**, *208*, 2005 – 2016; b) E. D. Mortenson, Y. X. Fu, *Curr. Pathobiol. Rep.* **2013**, *1*, 37 – 42.
[15] a) H. Oka, Y. Emori, O. Ohya, N. Kobayashi, H. Sasaki, Y. Tanaka, Y. Hayashi, K. Nomoto, *Immunol. Lett.* **1999**, *70*, 109 – 117; b) M. R. Shurin, L. Lu, P. Kalinski, A. M. Stewart-Akers, M. T. Lotze, *Springer Semin. Immun.* **1999**, *21*, 339 – 359.
[16] J. M. Brewer, M. Conacher, C. A. Hunter, M. Mohrs, F. Brombacher, J. Alexander, *J. Immunol.* **1999**, *163*, 6448 – 6454.
[17] a) D. T. O'Hagan, E. De Gregorio, *Drug Discovery Today* **2009**, *14*, 541 – 551; b) A. Pashine, N. M. Valiante, J. B. Ulmer, *Nat. Med.* **2005**, *11*, S63 – S68.
[18] a) J. Conniot, J. M. Silva, J. G. Fernandes, L. C. Silva, R. Gaspar, S. Brocchini, H. F. Florindo, T. S. Barata, *Front. Chem.* **2014**, *2*, 105; b) R. E. Serda, *Int. J. Nanomed.* **2013**, *8*, 1683 – 1696; c) A. L. St. John, C. Y. Chan, H. F. Staats, K. W. Leong, S. N. Abraham, *Nat. Mater.* **2012**, *11*, 250 – 257; d) J. Kim, W. A. Li, Y. Choi, S. A. Lewin, C. S. Verbeke, G. Dranoff, D. J. Mooney, *Nat. Biotechnol.* **2015**, *33*, 64 – U241; e) J. Park, S. H. Wrzesinski, E. Stern, M. Look, J. Criscione, R. Ragheb, S. M. Jay, S. L. Demento, A. Agawu, P. L. Limon, A. F. Ferrandino, D. Gonzalez, A. Habermann, R. A. Flavell, T. M. Fahmy, *Nat. Mater.* **2012**, *11*, 895 – 905.
[19] E. Phillips, O. Penate-Medina, P. B. Zanzonico, R. D. Carvajal, P. Mohan, Y. P. Ye, J. Humm, M. Gonen, H. Kalaigian, H. Schoder, H. W. Strauss, S. M. Larson, U. Wiesner, M. S. Bradbury, *Sci. Transl. Med.* **2014**, *6*, 260ra149.

Received: July 6, 2015
Revised: August 25, 2015
Published online: September 25, 2015

