LETTER

Medicinal Surface Modification of Silicon Nanowires: Impact on Calcification and Stromal Cell Proliferation

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ABSTRACT Medicinal surface modification of silicon nanowires (SiNWs) with selected bisphosphonates, such as the known antiosteoporotic drug alendronate, is described. In terms of specific assays relevant to orthopedic applications, the impact of selected bisphosphonate attachment on acellular calcification in simulated plasma is reported. To further investigate biocompatibility, proliferation assays of these modified nanowires were carried out using an orthopedically relevant cell line: mesenchymal stem cells derived from mouse stroma. It is found that the identity of the bisphosphonate ligand strongly and sensitively impacts its resultant cytotoxicity.

KEYWORDS: nanowires • calcium phosphate • bisphosphonates • tissue engineering • mesenchymal cells

One-dimensional semiconducting nanowires are proving to be a significant class of active nanostructures (1). Among them, Si nanowires (Si NWs) in particular have been the focus of intense interest due to their potential applications in the field of nanophotonics (2), biological/chemical sensors (3), and molecular electronics (4−6). In more recent examples, impressive advances have been recognized in sensing events with single viral particles (7) and communication with individual neurons (8). While much of these gains have explored the wires' semiconducting relevance to information storage or sensing events, it is perceived that semiconductor nanowires should also be considered for roles in active, selective therapeutic responses in diseased or injured tissue.

Nanostructured silicon's utility as a biomaterial has been greatly amplified by reports of facile calcium phosphate growth on the surface of porous Si in the presence (9) or absence (10, 11) of electrical bias, suggesting that silicon itself could be an important bioactive material. With specific attention to SiNWs, previous work from our laboratories demonstrated the ability of SiNWs to facilitate the growth of uniform synthetic bone coatings along their surface and to support the facile proliferation of fibroblast cells in their presence (12), indicating that SiNWs would be ideal candidates for orthopedic processes requiring the ability to promote bone regeneration within the body. In assessing various aspects of the biorelevance of this type of nanowire, other groups have subsequently shown the role of SiNW orientation on cytocompability (13) and that of nanowire surface oxide on altering common enzymatic reactions in vitro (14, 15).

Surface chemistry is a key issue in developing new orthopedic biomaterials. One perceived advantage in the possible use of nanowires in this type of application lies not only with the diverse surface functionalities that are possible with this vector but also with the density of such moieties. As candidates, bisphosphonates such as alendronate are widely used for the treatment of a variety of bone diseases characterized by excessive bone resorption (16), including tumor-induced hypercalcemia (17), Paget's disease (18), and osteoporosis (19). They have a strong affinity for calcium phosphate, the major mineral component of bone, and hamper osteoclastic bone resorption by inhibiting the key enzyme farnesyl pyrophosphate synthetase (20, 21). Recently, the therapeutic value of bisphosphonates has also been expanded by the discoveries that alendronate is effective in the prevention of urolithiasis by inhibition of calcium stone formation (22), as well as inhibiting invasion of PC-3 prostate cancer cells by affecting the mevalonate pathway (23). Therefore, evaluating the biocompatibility of calcium phosphate coated SiNWs (CaP/SiNWs) and bisphosphonate modified CaP/SiNWs composites is a valuable part of designing new effective orthopedic biomaterials if a permanent semiconducting conduit for bone regeneration is desired.

In this work, we present a detailed study on the calcification behavior of SiNWs under various surface treatments, as well as a straightforward method of coupling bisphosphonates onto the CaP/SiNW surface. Proliferation assays of mouse stromal cells in the presence of these composites were performed in order to evaluate their biocompatibilities. As the dose-dependent nature of alendronate with regard to cytotoxicity is well established (24), it was then decided to also evaluate the effect of replacing the exposed primary amine of alendronate with an alternate moiety; given the

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Received for review December 5, 2008 and accepted December 19, 2008
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DOI: 10.1021/am800219r

Published on Web 12/30/2008

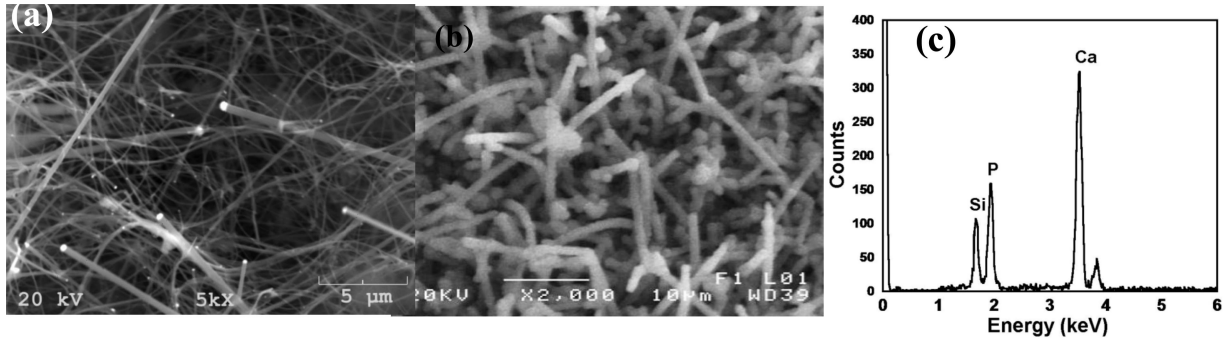

FIGURE 1. (a) SEM image of as-prepared SiNWs (scale bar 5 μm). (b) SEM image of calcium phosphate coated SiNWs (scale bar 10 μm). (c) EDX spectrum associated with image (b), showing calcium and phosphorus signals (along with Si).

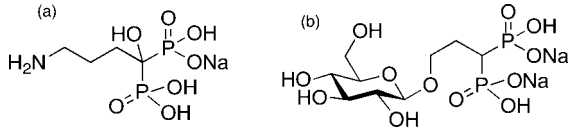

FIGURE 2. Structures of (a) alendronate and (b) the glucose bisphosphonate conjugate.

prevalence of glycoproteins on cell surfaces, a new glucose-bisphosphonate compound was prepared and subsequently coupled to the calcified SiNW surface to further assess the dependence of functional group identity on cell proliferation of these mesenchymal stem cells.

The role of surface chemistry on biomineralization in these nanowires was initially assessed by acellular calcification assays induced electrochemically (9). Typical experiments were carried out on SiNWs prepared by a VLS route (12) with an average diameter of approximately 160 nm and lengths in the tens of micrometers range (Figure 1a). In these calcification assays, a cell with SiNWs attached to a graphite cloth (or Si wafer) served as the working electrode, platinum foil was used as the counter electrode, and simulated body fluid (SBF) solution acted as the electrolyte. SBF has ion concentrations nearly equal to those of human plasma and is a well-established screen for evaluating the suitability of a particular material for orthopedic purposes (25). In this work, a cathodic bias was applied to SiNWs with the current density varying from 5 to 10 mA/cm^2 and duration of bias ranging from 60 to 120 min. Early work established that it is necessary to seed the Si nanowire surface with a finite amount of Ca^{2+} ions in order to induce calcification in vitro, presumably to achieve a critical density of nucleation centers for precipitation of the target calcium phosphate phases (12). Samples were then soaked in SBF for varying time periods at 37 °C under zero bias, with calcification evaluated in a given sample (after DI water rinse and drying) by SEM and EDX.

Nitrogen-containing bisphosphonates such as alendronate have a high affinity to calcified bone phases (26). Therefore, the surface modification of calcium phosphate coated SiNWs (Figure 1b) with alendronate (Figure 2a) was achieved by a facile immersion of CaP/SiNWs in a 2.5 mM aqueous alendronate solution at room temperature for 24 h. To probe coverage of the alendronate species on the calcium phosphate coating in a qualitative fashion, we coupled fluorescein isothiocyanate (FITC) to the exposed primary amine groups at the surface of alendronate modified CaP/SiNWs by simple immersion in an aqueous 1 mM FITC solution at 4 °C overnight. The samples were then rinsed with DI water to remove any surface absorbed FITC residues and characterized by fluorescence microscopy. Figure 3 shows a fluorescence image and associated spectrum of a labeled alendronate modified CaP/SiNWs surface. The green structures (marked by arrows) were FITC labeled nanowires. The green shell represents the calcium phosphate layer coupled with alendronate. The stable, strong green emission at 525 nm indicates that the alendronate has been successfully coupled to the surface of calcium phosphate coated SiNWs in a relatively uniform fashion. Control experiments with nonalendronate treated NWs produced structures whose green emission can be eliminated with repeated solvent washings (not shown).

To investigate the impact of alendronate on the calcification of Si NWs, this specific bisphosphonate was introduced at different stages of the calcification assay described above. To achieve this, a modified SBF solution containing 2.5 mM alendronate was prepared for use as electrolyte and soaking medium. For experiment 1, cathodic bias was applied to SiNWs in the alendronate-modified SBF solution, followed by soaking the samples in regular SBF. In experiment 2, SiNWs were biased in regular SBF, immersed in 2.5 mM aqueous alendronate solution at room temperature for 24 h, and then soaked in regular SBF solution for an extended period (up to 4 weeks). Finally, for experiment 3, SiNWs were cathodically biased in regular SBF solution and then soaked in alendronate-modified SBF solution (also for extended periods). Interestingly, it was found that in all of these experiments involving the presence of alendronate there was no detectable calcification of SiNWs at the time scales typically measured (1−4 weeks). This inhibition of SiNW calcification in the presence of alendronate is likely a consequence of the strong affinity of alendronate for any exposed calcium centers, which leads to a significant suppression of necessary nucleation sites for precipitation of calcium phosphate from SBF solution under zero bias.

Another complementary, yet necessary, step in evaluating a new material for biomedical applications is to study its cytocompatibility via a cell-based assay. While previous studies showed a lack of cytotoxicity for SiNWs to fibroblasts (12), it is essential to evaluate the behavior of a more

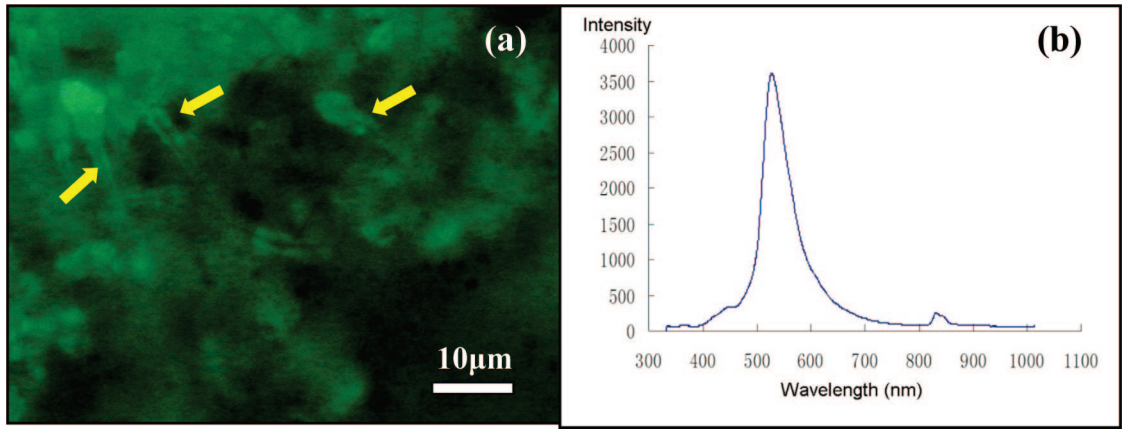

FIGURE 3. (a) Fluorescent image of calcium phosphate coated Si NWs modified with alendronate and coupled with FITC. (b) Fluorescence spectrum of this sample.

orthopedically relevant cell line when exposed to these surface-modified SiNWs. One such candidate is mouse stromal cells derived from bone marrow, capable of differentiation into osteoblasts (27), chondrocytes, and adipocytes under the appropriate stimulus and environment (28). When injected systemically into tissue, these cells have been shown to localize to the tissue of interest and may be used to treat disorders such as osteoporosis to effect bone growth augmentation or bone repair (28). Therefore, an investigation of how modification of the SiNW surface chemical composition affects the proliferation of this type of cell line is an important assessment of the potential impact of such nanomaterials on bone repair. With specific regard to alendronate, while the phosphonate groups provide strong anchors to the apatite surface, previous studies have suggested that the primary amine moiety plays a major role in its farnesyl transferase inhibitory activity, with a dose-dependent sensitivity to cytotoxicity (24). Thus, in considering other possible candidates for evaluation, the prevalence of glycoproteins on cell surfaces and their favorable biological response was taken into account; hence, a new glucose-bisphosphonate compound (Figure 2b) was prepared for coupling to the calcified SiNW surface, thereby providing further assessment of the dependence of functional group identity on its suitability. Full preparative details and characterization data for this compound, disodium [(2,3,4,6-tetra-*O*-hydroxyl-β-D-glucopyranosyl)propyl]-1,1-bisphosphonate, can be found in the Supporting Information.

Before the cells were plated into the well, the materials were sterilized in 70% ethanol and sterilized DI water for 24 h and 5 min, respectively. After they were dried, the materials were exposed to UV light for 2 h. A total of 10 000 mouse stromal cells (D1-ORL-UVA; ATCC number CRL-12424) were cultured per well in the presence of SiNWs, calcium phosphate coated SiNWs, alendronate modified CaP/SiNWs, and glucose bisphosphonate modified CaP/SiNWs (~200 μg of nanowires per well). The number of cells was counted at days 3, 5, and 7. The results of this assay are shown in Figure 4, and the relative cytocompatibility of these nanowires follows the following order: glucose bisphosphonate CaP/SiNWs ≈ CaP/SiNWs ≈ SiNWs ≫ alendronate CaP/SiNWs. Several conclusions may be drawn as a

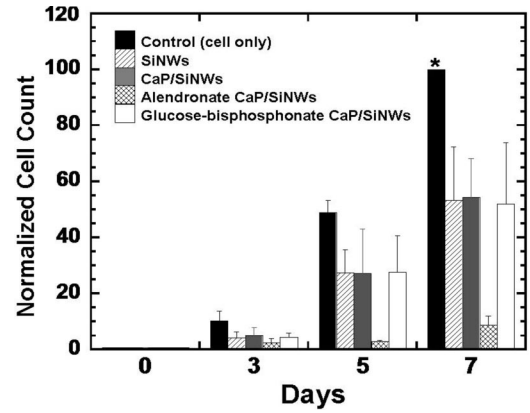

FIGURE 4. Graph of mouse stromal cell proliferation demonstrating the noncytotoxic behavior of SiNWs, CaP/SiNWs, and glucose bisphosphonate CaP/SiNWs, as well as the cytotoxic behavior of alendronate CaP/SiNWs. All cell counts were normalized with respect to the number of cells in the control group after 7 days of proliferation (100%).

result. In terms of tolerance of surface coatings, there is no significant difference observed between the behavior of the calcium phosphate coated SiNWs and that of the as-prepared silicon oxide terminated SiNWs, consistent with cell surface interactions in each case with an oxophilic substrate. Introduction of the strongly bound alendronate with the exposed primary amine, producing a strong cytotoxic response, is quite consistent with our a priori expectations from a toxicity perspective; also, if we take into account the observed inhibition of calcification in the modified SBF assays, these results overall are consistent with the in vivo results of Bode and co-workers, who found that the presence of alendronate in synthetic bone cement did not increase bone formation in femoral defects present in a rabbit model (29). This response is readily "switched off", however, when the primary amine containing alendronate derivative is subsequently replaced by the cytocompatible glucose bisphosphonate/calcified SiNW species. Not surprisingly, then, it is clear that the functional group identity of these bisphosphonate species plays a major role in influencing the overall biological response in this type of biomaterial.

In conclusion, this work demonstrates a detailed study of the impact of surface composition on the calcification of SiNWs and the subsequent coupling of selected bisphospho-

nates onto their surface. The alendronate modified CaP/SiNWs exhibit a cytotoxic behavior that is consistent with its pharmacological mode of action in vivo; deliberate subtle modification of the exposed primary amine with glucose sensitively improves the cytocompatibility of the nanowire vector. On the basis of these results, an expansion of the range of therapeutic tunability of these nanowires (for orthopedics and beyond) is now envisioned.

Acknowledgment. J.L.C. gratefully acknowledges the Robert A. Welch Foundation for support of this research. J.-L.M. and Y.B. thank the National Institute of General Medical Sciences/NIH (1R01 GM067610) for financial support.

Supporting Information Available: Text and a figure giving experimental details for the synthesis of disodium [(2,3,4,6-tetra-O-hydroxyl-β-D-glucopyranosyl)propyl]-1,1-bisphosphonate, along with the composition of simulated body fluid (SBF) used in acellular calcification assays and protocols utilized for the cell proliferation experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

(1) Xia, Y.; Yang, P.; Sun, Y.; Wu, Y.; Mayers, B.; Gates, B.; Yin, Y.; Kim, F.; Yan, H. *Adv. Mater.* **2003**, *15*, 353.
(2) Gudiksen, M. S.; Lauhon, L.; Wang, L.; Smith, D.; Lieber, C. M. *Nature* **2002**, *415*, 617.
(3) Cui, Y.; Wei, Q.; Park, H.; Lieber, C. M. *Science* **2001**, *293*, 1289.
(4) Cui, Y.; Lieber, C. M. *Science* **2001**, *291*, 891.
(5) Huang, Y.; Duan, X.; Wei, Q.; Lieber, C. M. *Science* **2001**, *291*, 630.
(6) Huang, Y.; Duan, X.; Cui, Y.; Lauhon, L.; Kim, K.; Lieber, C. M. *Science* **2001**, *294*, 1313.
(7) Patolsky, F.; Zheng, G.; Hayden, O.; Lakadamyali, M.; Zhuang, X.; Lieber, C. M. *Proc. Natl. Acad. Sci.* **2004**, *101*, 14017.
(8) Patolsky, F.; Timko, B.; Yu, G.; Fang, Y.; Greytak, A. B.; Zheng, G.; Lieber, C. M. *Science* **2006**, *313*, 1100.
(9) Canham, L. T.; Newey, J. P.; Reeves, C. L.; Houlton, M. R.; Loni, A.; Simon, A. J.; Cox, T. I. *Adv. Mater.* **1996**, *8*, 847.
(10) Canham, L. T. *Adv. Mater.* **1995**, *7*, 1033.
(11) Canham, L. T.; Reeves, C. L.; King, D. O.; Branfield, P. J.; Crabb, J. G.; Ward, M. C. L. *Adv. Mater.* **1996**, *8*, 850.
(12) Nagesha, D. K.; Whitehead, M. A.; Coffer, J. L. *Adv. Mater.* **2005**, *17*, 921.
(13) Kim, W.; Ng, J. K.; Kunitake, M. E.; Conklin, B. R.; Yang, P. *J. Am. Chem. Soc.* **2007**, *129*, 7228.
(14) Qi, S.; Yi, C.; Chen, W.; Fong, C.; Lee, S.-T.; Yang, M. *ChemBioChem* **2007**, *8*, 1115.
(15) Yi, C.; Fong, C.; Chen, W.; Qi, S.; Lee, S.-T.; Yang, M. *ChemBioChem* **2007**, *8*, 1225.
(16) Fleisch, H. *Osteroporosis Int.* **1993**, *3*, 15.
(17) Coleman, R. E. *Cancer Treat. Rev.* **2001**, *27*, 165.
(18) Roux, C.; Dougados, M. *Drugs* **1999**, *58*, 823.
(19) Delmas, P. D. *Lancet* **2002**, *359*, 2018.
(20) Ito, M.; Chokki, M.; Ogino, Y.; Satomi, Y.; Azuma, Y.; Ohta, T.; Kiyoki, M. *Calcif. Tissue. Int.* **1998**, *63*, 143.
(21) Rogers, M. J.; Gordon, S.; Benford, H. L.; Coxon, F. P.; Luckman, S. P.; Monkkonen, J.; Frith, J. C. *Cancer* **2000**, *88*, 2961.
(22) Senzaki, H.; Yasui, T.; Okada, A.; Ito, Y.; Tozawa, K.; Kohri, K. *Urol. Res.* **2004**, *32*, 223.
(23) Virtanen, S. S.; Vaananen, H. K.; Harkonen, P. L.; Lakkakorpi, P. T. *Cancer Res.* **2002**, *62*, 2708.
(24) Giuliani, N.; Pedrazzoni, M.; Negri, G.; Passeri, G.; Impicciatore, M.; Girasole, G. *Bone* **1998**, *22*, 455.
(25) Kokubo, T.; Kushitrani, H.; Sakka, S.; Kitsigi, T.; Yamamuro, T. *J. Biomed. Mater. Res.* **1990**, *24*, 721.
(26) Rodan, G. A.; Balena, R. *Ann. Med.* **1993**, *25*, 373.
(27) Whitehead, M. A.; Fan, D.; Mukherjee, P.; Akkaraju, G. R.; Canham, L. T.; Coffer, J. L. *Tissue Eng. A* **2008**, *14*, 195.
(28) Tuan, R. S.; Boland, G.; Tuli, R. *Arthritis Res. Ther.* **2003**, *5*, 32.
(29) Bodde, E. W. H.; Kowalski, R. S. Z.; Spauwen, P. H. M.; Jansen, J. A. D. D. S. *Tissue Eng. A* **2008**, *14*, 29.

AM800219R