Nanofiber Catalyst Production Using Anaerobic Bacteria

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Abstract:

The goal of this research was to carry out comparative studies on nanowires fabricated using nanoparticle (NP) catalysts from several different strains of bacteria. We identified and selected four NP-producing candidates: magnetotactic bacteria strains MS-1, MV-1, and MC-1, which produce magnetite (Fe_3O_4) NPs; and a strain of sulfate-reducing bacteria (SRB), *Desulfovibrio gigas*, which may produce ZnS NPs. The size and shape of these catalysts affect the diameter and cross section of the nanowires, which are important in potential nanoelectronic applications. We also plan to test zinc oxide nanowires in a nano-biosensor for detection of molecules tagged with green fluorescent protein.

Since formation of ZnS NPs has only been documented for mixed natural cultures of SRB, an initial study examined media that might stimulate ZnS NP growth in *D. gigas*. Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) were not yet able to detect the presence of ZnS particles.

Introduction:

Miniaturization goals in electronics will require new, cost-efficient, highly-reproducible methods for producing nano-scale devices, of which nanowires (NW) and carbon nanotubes (CNTs) are integral components. Controlling diameter, shape, orientation, and alignment of NW/CNTs is important to researchers. One approach is production of NP catalysts via self-regulating biological systems. Four anaerobic strains of bacteria, requiring complex culturing techniques, were selected to produce NPs of unique size and shape because these variables should directly influence the attributes of subsequently grown nanofibers. The three magnetotactic strains also have potential to aid in lateral alignment of the NPs (and thus the nanofibers).

D. gigas was selected because it was readily available and was one of the species included in the natural, mixed SRB culture study in which ZnS NPs were successfully produced [1]. Our group previously produced NPs using MS-1, the easiest of the four strains to culture, for ZnO nanowires growth.

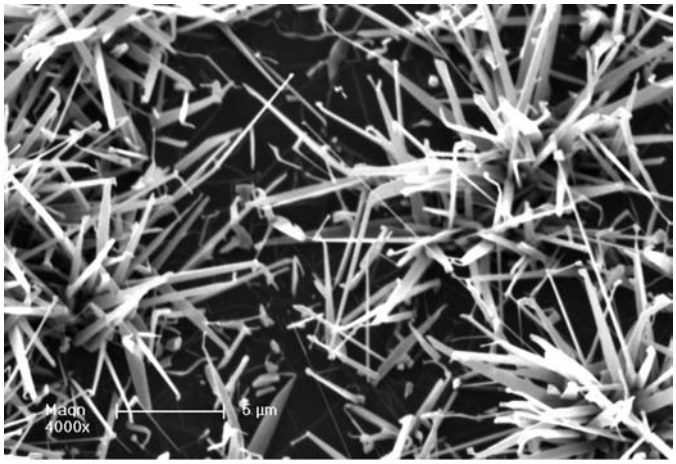

Figure 1: MS-1 NP-catalyzed ZnO nanowires (SEM, 4000x magnification).

Materials and Methods:

The MV-1 and MC-1 bacteria were obtained from Dr. Dennis A. Bazylinski (Department of Microbiology, Iowa State University). Samples from the concentrated liquid cultures of MV-1 were refrigerated in epitubes for use in initiating new cultures and CNT growth. MC-1 cannot be refrigerated, thus samples were isolated for immediate CNT growth. The remainder of both bacterial solutions was cryopreserved at -80ºC in an 80% glycerol solution.

Growth media for a live liquid culture of MV-1 was prepared using an as of yet unpublished recipe received from Dr. Bazylinski's lab. Preparation required sparging with N_2 gas, use of an anaerobic chamber for addition of several ingredients, and replacing the headspace with N_2O gas. Media and components were autoclaved (liquid cycle, 20 minutes). Remaining components were added anaerobically and aseptically, then the media was refrigerated until inoculation.

D. gigas was obtained from Dr. Christopher House (Department of Earth and Geological Sciences, Pennsylvania State University). Growth Media 149 and Media 63 (http://DSMZ.de) were used. Both

formulations were altered to create an environment promoting ZnS formation by the addition of $ZnSO_4$. Basicity of the media (pH ~7.5) caused $Zn(OH)_2$ precipitation. Media 63 pH was decreased to ~ 6.3 to solublize the precipitate, but the pH of Media 149 was retained at 7.8. A 20%N2/80%CO_2 mix (0.5 bar) comprised the headspace gas for both media. Five samples of each media, with Zn concentrations ranging from 0 to 0.01 g/mL, were inoculated with *D. gigas* and allowed to incubate unshaken at 30ºC for 4 days.

Results and Conclusions:

MV-1 and MC-1 cultures were printed onto silicon wafers and ignited (800ºC, 1 hour) in preparation for CNT growth. Atomic force microscopy revealed successful CNT formation on the MC-1 sample wafer. However, the MV-1 bacteria cells did not lyse and disintegrate as expected, and nanofiber catalysis was unsuccessful. Although we cannot explain this unexpected behavior, salt build-up on the surface of the cells was suspected to have contributed to this phenomenon.

Presence of *D. gigas* growth and particle formation could not be visually assessed for 4 out of 5 Media 149 samples and high-zinc Media 63 samples due to precipitation of $Zn(OH)_2$. Growth was visually confirmed in both samples containing no Zn. Media 63 samples containing 0.01 g/mL $ZnSO_4$ and no Zn were placed on silicon wafers and ignited for SEM/EDS analysis. No particles were observed on either wafer using SEM. This could have resulted from limitations in the resolution of this technique. Significant presence of zinc and sulfur could not be confirmed in either sample by EDS. We suspect that this failure to obtain ZnS NPs using *D. gigas* can be reversed by using a lower Zn concentration. Exposing NPs to conditions for nanowire growth could also aid in detection.

Future Work:

Further study requires availability of these bacteria cultures. Thus, an immediate goal is to sustain a living culture of each strain, starting from the cryopreserved stock cultures. In the future, we want to use a magnetic field to laterally align magnetotactic cultures prior to printing. Also, CNTs catalyzed from each particle type need to be characterized (i.e. number of walls, diameter). The ZnS NPs represent a potential for much smaller wire formation. Experiments will focus on identifying culture conditions that could facilitate formation of these particles from *D. gigas*. Alteration of Zn concentration and pH is a starting point. More sophisticated strategies of feeding Zn to avoid toxicity yet facilitate NP formation could be tested. Finally, since the utility of these ZnS NPs is still exploratory, attempting to isolate these NPs from mixed culture conditions where they have been reported [1] may prove worthwhile in establishing their utility, thus warranting the effort of developing a production strategy.

Acknowledgements:

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References:

[1] Labrenz, et al., Formation of Sphalerite (ZnS) Deposits in Natural Biofilms of Sulfate-Reducing Bacteria. Science, 290, 1744-1747 (2000).

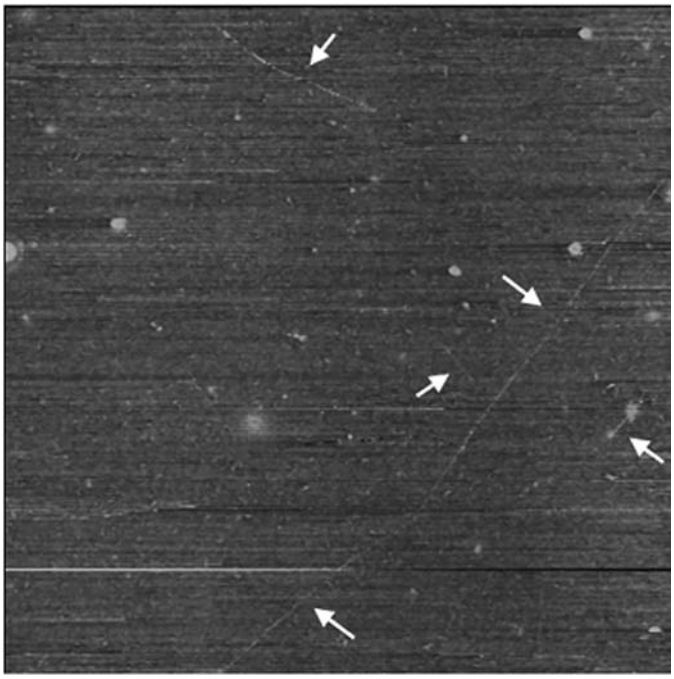

Figure 2: 7-by-7 µm AFM image of CNTs on MC-1 NPs, indicated by white arrows.