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

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Statement of Purpose: The utility of nanostructured silicon as a biomaterial has been greatly amplified by reports of facile calcium phosphate growth on the surface of porous Si, suggesting that silicon itself could be an important bioactive material. With specific attention to silicon nanowires (SiNWs), our previous work demonstrated their ability to facilitate the growth of uniform synthetic bone coatings along their surface and to support the facile proliferation of fibroblast cells in their presence, indicating that SiNWs could be ideal candidates for orthopedic processes requiring the ability to promote bone regeneration within the body.

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 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. Therefore, evaluating the biocompatibility of calcium phosphate coated SiNWs (CaP/SiNWs) and bisphosphonate modified CaP/SiNWs composites on a more orthopedically-relevant cell line is a valuable part of designing new effective orthopedic biomaterials.

Methods: Si NWs were synthesized from the pyrolysis of silane at 600^{o}C for 20 min, using Au as the catalyst via a vapor-liquid-solid mechanism. The calcification of SiNWs was initiated by cathodic bias in simulated body fluid (SBF) with the applied current density varied from 5 to 10 mA/cm^2 and duration varied from 60 to 120 min. Samples were then soaked in SBF for varying time periods at 37^{o}C under zero bias, with calcification evaluated then by SEM and EDX. The surface modification of calcium phosphate coated SiNWs (CaP/SiNWs) with bisphosphonates was achieved by a facile immersion of CaP/SiNWs in a 2.5mM aqueous bisphosphonates solution at room temperature for 24 hours. Alendronate was introduced into the calcification process at different stages to investigate its effect on the calcification. For the cell proliferation studies, 10,000 mouse stromal cells (D1-ORL-UVA; ATCC number CRL-12424) were cultured per well in the presence of SiNWs, CaP/SiNWs, alendronate modified CaP/SiNWs, and glucose-bisphosphonate modified CaP/SiNWs. The number of cells was counted at days 3, 5, and 7.

Results: All of the experiments involving the presence of alendronate resulted in 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. In terms of cytocompatibilities of surface coatings, there is no significant difference observed between the behaviors of the calcium phosphate coated SiNWs and that of the as-prepared oxide terminated SiNWs. 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 Bodde 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. This response is readily "switched off" however when the primary amine-containing alendronate derivative is subsequently replaced by a cytocompatible glucose-bisphosphonate / calcified SiNW species. Not surprisingly, then, it is clear that functional group identity of these bisphosphonate species plays a major role in influencing the overall biological response in this type of biomaterial.

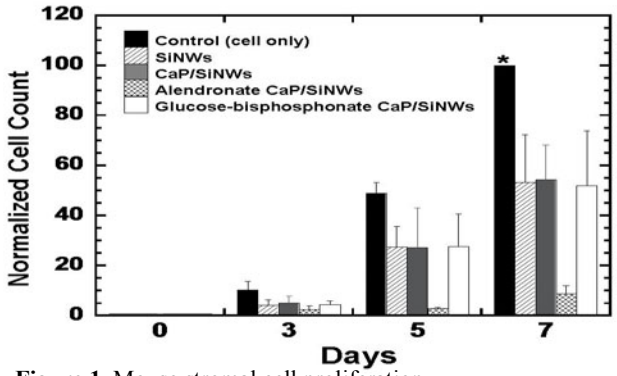

Figure 1. Mouse stromal cell proliferation.

Conclusions: This work provides a detailed study of the impact of surface composition on the calcification cytocompatibility of SiNWs,. The alendronate modified CaP/SiNWs exhibit a cytotoxic behavior that is consistent with its pharmacological mode of action *in vivo*; deliberate replacement of the exposed primary amine with glucose moieties sensitively improves the cytocompatibility of the nanowire vector. Based on these results, an expansion of the range of therapeutic tunability of these nanowires (for orthopedics and beyond) is now envisioned.

References:

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