Anti-cancer and Anti-microbial Selenium Nanoparticle Bone Scaffolds

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Statement of Purpose: Osteosarcoma is a highly-metastatic bone cancer that is a rare disease (only occurring in 2% of adults, but up to 4 and 7% of children and teens, respectively).[1] It is a highly aggressive disease with a high risk of recurrence. After limb-sparing resection and surgery, there is the additional risk of implant infection, loosening and other failure. Here, we employed the deposition of selenium nanoparticles (SeNP) as a means to create a nano-scale topography on a polymer as well as adding a material with antibacterial[2] and anticancer[3] characteristics with minimal effects on healthy mammalian cell cultures.[4] Results showed that increased concentrations of SeNP resulted in a greater reduction in osteosarcoma proliferation while increasing the osteogenic activity of healthy osteoblasts.

Methods: Poly(l-lactic acid) (PLLA) was used as a foundation substrate, and was prepared by dissolving PLLA pellets in chloroform and cast onto glass dishes to generate the base of the scaffold. SeNP were generated by mixing 3ml each of 0.1M glutathione (GSH) and 0.025M Na_2SeO_3. To precipitate SeNP, 2M NaOH was added to bring the solution to the alkaline range (pH > 8) and diluted in DiH_2O to stop the reaction. To modify the SeNP dosage, the PLLA films were incubated in the GSH/Na_2SeO_3 step (T_1) or the NaOH step (T_2) for, 5, 30 or 60 s. A sample incubated for T_1=30 and T_2=5 would, thus, be referred to as 30:5. Each sample was characterized via SEM, AFM and goniometry to determine SeNP size and coverage, surface nanoroughness and surface energy. Samples were sterilized in EtOH for 1 hr and rinsed thoroughly with sterile PBS prior to in vitro assays. Each disc was seeded with either 5,000 MG-63 osteosarcoma cells (ATCC CRL-1427), or HfOB human osteoblasts (ATCC CRL-11372). The plates were incubated for two days, and cell viability was determined via an MTS assay. Alkaline Phosphatase activity of osteoblasts was monitored via the Quantichrome ALP Assay Kit (Bioassays) for 2, 7 and 14 days after seeding. All experiments were conducted in triplicate and repeated three times each.

Results and Discussion: As expected, with an increase in development time, more selenium nanoclusters formed on PLLA. For example, there was a fourfold increase in coverage from 1 min to 5 min of development time with little change in SeNP size. Additionally, with increased selenium—and, thus, an increase in nanoroughness—osteosarcoma cells were less capable of adhering and proliferating on the bare PLLA discs (Fig. 1A). Although more experimentation is required, the present study indicates that PLLA can be easily transformed to keep bone cancer cells from returning after a bone tumor is resected. PLLA is already commonly used in bone tissue engineering applications and this study highlights for the first time that through a selenium coating, it can be easily used in bone cancer applications.

Conclusions: Nanoparticle synthesis using the described method resulted in evenly-coated substrates with similarly-sized SeNP on each. NaOH exposure, T_2, seemed to have more control over the coating process, resulting in the greatest changes in surface energy, nanoroughness and number of particles per area. However, the coverage of the substrate seemed to saturate between 30 and 60 s, suggesting that there is a limit to the SeNP concentration in these coatings (Figure 1B). Additionally, at these longer times, cellular responses were insignificantly changed in both cancer cytotoxicity (30:30 vs 30:60 or 60:30 in Fig. 1A) and ALP activity (data not shown).

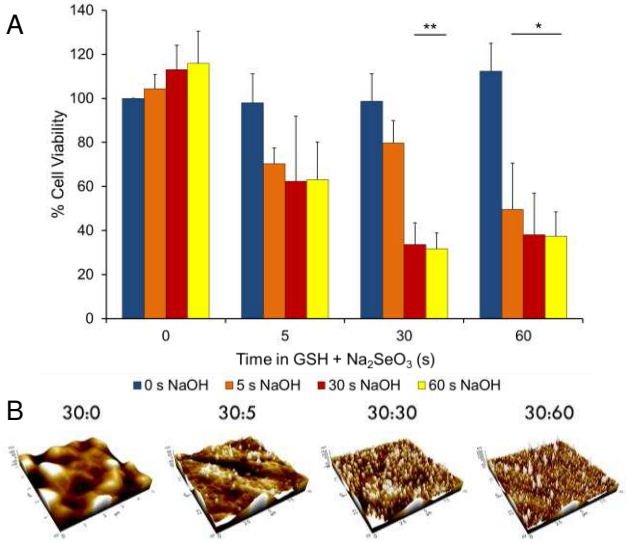

Figure 1. A: Osteosarcoma (MG-63) cells were seeded onto Se-PLLA films and grown for 2 days. n=3, N=4 *p<0.05, **p<0.01 compared to bare PLLA (0:0) under same conditions. Data = mean ± SEM. **B:** AFM of samples treated for T_1 = 30 s and varied T_2.

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