Creating Bone-Promoting, Cancer-Inhibiting, Bacterial-Inhibiting Implants Using Selenium Nanoclusters

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Introduction: Two common causes leading to bone implant failure are: an insufficient prolonged bonding between the implant and the surrounding bone as well as infection. For patients who receive orthopedic implants after cancerous tissue is removed, it would also be beneficial to implant an anti-cancer material that can impede the return of cancerous tissue growth that may develop from cancer cells not removed during surgery. Therefore, the objective of this in vitro study was to create a coating material that can: (i) promote healthy, normal bone growth; (ii) inhibit bacterial attachment and (iii) impede cancer growth. To achieve that objective, conventional orthopedic implant materials (such as titanium (Ti) and stainless steel (SS)) were coated with selenium (Se) nanoclusters. Different coating densities were achieved by varying Se concentration in the reaction mixture. Se nanocluster coatings formed on all materials mentioned and were shown to enhance healthy osteoblast (bone-forming cell) and inhibit cancerous osteoblast proliferation in either separate-culture experiments or co-culture experiments. Functions of *S. epidermidis* (one of the leading bacteria that infect implants) were inhibited on all materials coated with Se-nanoclusters compared to uncoated materials.

Methods: The Se coating process was described in [1]. Briefly, Ti substrates and SS substrates (Alfa Aesar) were exposed to 4:1 molar mixtures of glutathione (GSH, TCI) and sodium selenite (Alfa Aesar) followed by the addition of 1M NaOH to bring the pH into the alkaline regime. Three different solution concentrations of Se (0.42mM, 0.84mM and 1.68mM) were used to achieve different coating doses (Low-nSe, Medium-nSe and High-nSe, respectively). Surfaces substrates were visualized using a scanning electron microscope.

Primary human calvarial osteoblasts (ScienCell Research Labs) and mouse osteosarcoma osteoblasts (ATCC) were used for separate-culture and co-culture experiments. Briefly, separate-culture experiments were conducted according to standard protocols [1] for 3 days. Co-culture experiments were implemented by co-seeding healthy osteoblasts (pre-stained with a fluorescent dye DiO (Invitrogen)) and cancerous osteoblasts (pre-stained with a fluorescent dye DiD (Invitrogen)) on uncoated Ti and Se-coated Ti. The density of each type of cell was determined using fluorescent microscopy at 4, 17, 28, 40, 53 and 65 hrs. Bacteria assays were conducted according to standard protocols [2] and cell densities were determined via optical densities of the solution of the bacteria removed from substrates by vortexing and sonicating. Data is reported as mean ± SEM; N=3.

Results: Density of healthy cells increased while that of cancerous cells decreased on Se-coated Ti (compared to uncoated Ti) in separate-culture (Fig.1)

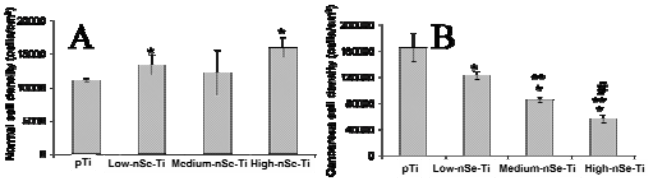

Fig.1. Increased healthy osteoblast densities (A) and decreased cancerous osteoblast densities (B) on Se-coated Ti after 3 days of culture. In 1A, * $p<0.05$ compared to uncoated Ti (pTi). In 1B, * $p<0.01$ compared to pTi; ** $p<0.01$ compared to Low-nSe-Ti; # $p<0.05$ compared to Medium-nSe-Ti.

In co-culture, healthy cells proliferated on High-nSe-Ti while cancerous cells did not grow on pTi (Fig.2)

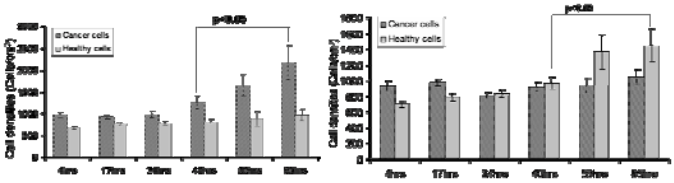

Fig.2. (Left) Increased cancerous osteoblast density after 53 and 65 hrs on uncoated Ti. There was no significant change of osteoblast densities on uncoated Ti. (Right) Increased healthy osteoblast density after 53 and 65 hrs on High-nSe-Ti. Cancerous osteoblasts did not show any significant growth on High-nSe-Ti.

Bacteria densities on Se-coated Ti were also decreased compared to uncoated Ti (Fig.3).

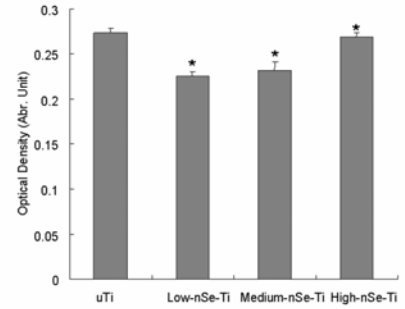

Fig.3. Decreased *S. Epidermidis* density on Se-coated Ti. *$p<0.05$ compared to uncoated Ti (uTi).

Conclusions: This study showed that Se nanocluster coatings can transform an orthopedic material into a bone-promoting, cancer-inhibiting, and bacteria infection-inhibiting implant. Further in vitro and in vivo research is recommended, on this promising new nano-coating material.

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References: [1]. P.A. Tran, et al., J Biomed Mater Res A. (2009) in press.
[2]. E. Taylor, et al., Int.J.Nanomedicine 2009:4 145–152