Nanofiber-Coated Surfaces affect the Proliferation and Differentiation of Osteoprogenitors in vitro
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Introduction: There are about 800,000 orthopedic reconstructive surgery procedures (primarily hip and knee joint replacements) and one million dental implants performed in the USA each year. However the optimal material for different implants in bone has not been determined. Modified or coated titanium and its alloys have become candidates for next generation implants. Nanofiber coated surface (NF) can be used to enhance osteoconduction. We hypothesized that coating surfaces with nanofibers would affect the proliferation and differentiation of osteoprogenitors to osteoblasts.

Materials and Methods: All NF’s were constructed at Nanosys, Inc. (Palo Alto, CA) to ~20-30 μm (+/−5 μm) in length. The NF catalyst seeding density was about 15 particles per μm². The diameters of the plain SiNF were 40 nm, oxidized SiNF were about 40 nm, and the TiO2 coated Si NF were about 60 nm. The TiO2 coating was applied by Atomic Layer Deposition (ALD) by Planar Systems, Inc (Fig. 1). The morphology of all the NF structures was a random “birds-nest” structure. MC3T3-E1 seeded on Ti alloy discs at a density of 2 x 105 cells/cm² (total 1.5 x 105) in 500 μl nonosteogenic media. Cultures were maintained for 42 days. QuantiChrom Alkaline Phosphatase assay Kit from Bioassay Systems, osteocalcin ELISA kit Biomedical Technologies Inc and picogreen dsDNA quantization assay kit from Invitrogen Inc. were used to measure ALP activity, osteocalcin and DNA respectively. One way ANOVA (Post hoc multi-comparisons with Tukey) was conducted using SPSS 14.0. Data were reported as mean ± standard error. A p value < 0.05 was chosen as the threshold of significance.

RESULTS: MC3T3-E1 cells proliferate on the surface of Titanium discs:
Each disk was seeded with 1.5 x 105 cells. After three days expansion in nonosteogenic media, cells on plastic surface (PS) reached confluence, followed by Ti alloy. Si NF and TiO2 NF had 10% of the original seeded cells while SiO2 NF had only 1% remaining. After 42-days culture in osteogenic media, PS surfaces still had the greatest number of cells, followed by Ti alloy and Si NF. Both SiO2 NF and TiO2 NF had very few cells. Regarding proliferation capacity after 42 days of culture, Si and SiO2 NF were much higher than Ti alloy and PS. The cell population on TiO2 NF did not increase after the 6 weeks cell culture (Table 1).

Table 1. The cell numbers on the five surfaces. Fold is the increase from day 0 to day 42, unit: 1000, n is 4–5. For day 0, * and †p < 0.05 vs. all remaining groups, For day 42, * and †p < 0.05 vs. all remaining groups, # p < 0.05 vs. group SiO2 and TiO2 NF. For fold, * p < 0.05 comparing day 0 and 42 of itself.

Discussion: In our experiments, cell attachment on nanofiber surfaces (Si, SiO2 and TiO2) was much lower than on titanium alloy surfaces without modification. There are several possible reasons for the low cell retention. First, the size of nanofibers used for coating may not be the optimal for MC3T3 cells. Second, the bulk physical surface of nanofiber coated coupons may not be conducive to cell adhesion due to the complex “birds nest” topography making it difficult for the cells to establish firm adhesive interactions. Third, the surface chemistry of the nanofibers may not be optimal. It is surprising that the cells on TiO2 nanofibers did not increase after 42 days in culture. The most plausible explanation is decreased cell proliferation on TiO2 nanofiber surfaces. Both differentiation markers (ALP-2 and OC) showed much higher values for cells on TiO2 nanofiber discs than all other groups including the plastic surface. The exact signaling mechanisms which initiate differentiation of osteoprogenitor cells on TiO2 surfaces in the present culture conditions are unknown and would be of great interest in future studies.

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Fig 1. Images of nanofibers on Ti alloy discs. i. SEM image of a Si NF coated surface with magnification 5,000x. Image taken with FESEM. ii. TEM image of one TiO2 coated Si nanofiber with magnification 110,000x, showing the composite structure of the TiO2 NF.

Fig 2. A: Ti alloy, B: Si NF, C: SiO2 NF, D: TiO2 NF, E: PS. i. The ALP-2 activity. * p < 0.05 vs group Ti alloy, Si NF and SiO2 NF. ii. Released OC in the media. * p < 0.05 vs group Ti alloy, Si NF, SiO2 NF and PS. iii. The profiles of the OC from day 0 to the end. one way ANOVA, n=4-5.

TiO2 NF had high OC per cell:
OC expression in the polystyrene group became evident at day 12 and increased rapidly thereafter. The release of OC in group Ti alloy, Si NF and TiO2 NF was much later (about day 24), but the amount in groups Ti alloy and TiO2 NF was in the same range as in group PS while group SiO2 NF was much lower (Fig. 2ii). At day 42, after adjustment for cell number, the OC per cell in group TiO2 NF was 25 times higher than other groups (p < 0.05). There was no OC detected in group SiO2 NF (Fig. 2ii).

For fold, * p < 0.05 comparing day 0 and 42 of itself.