Osteogenic Response of Stem Cells to Mineralized Silk Scaffolds and Calcitriol Supplement

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Objective

Our objective in this project is to determine the effects of mineralized silk and calcitriol on stem cell osteogenesis.

Introduction

Bone defects are a common occurrence in many clinical situations including high grade open fracture, high energy trauma, blast injuries, infection requiring debridement of bone, and resection of bone tumors(1). Current treatment options include autografts, allografts, and other synthetic materials which have significant limitations including inadequate size and shape, morbidity at the donor site, and a required second surgery(2). Because these solutions are not ideal, further research has shown that silk fibroin can be used as scaffold for bone regeneration due to its mechanical properties, biodegradability, and versatility in processing(2). With this, hydroxyapatite (HA) has also been shown to improve bonding of polymeric materials to bone and enhance cell adhesion and differentiation of osteoprogenitor cells(2). Bone is affected by many growth factors including bone morphogenic proteins (BMPs), transforming growth factor-β (TGF-β), insulin-like growth factors I and II (IGF-I and IGF-II), platelet-derived growth factor (PDGF), and acidic and basic fibroblast growth factor (aFGF and bFGF)(3). More specifically recombinant human bone morphogenetic proteins (rh-BMPs) have shown success in bone regeneration due to their ability to promote cell growth and differentiation and recruit osteoprogenitor cells that are essential for bone development, remodeling, and repair(4). These osteoinductive factors often require supraphysiological dosages to produce the necessary results for large defects. Calcitriol is the bioactive form of vitamin D and has been indicated as a supplement to improve bone homeostasis(5) and as an inductive factor *in vitro* for stem cell differentiation(6). Our study examines the synergistic effect of a mineralized silk scaffold and calcitriol on the osteogenic response of stem cells.

Methods

Silk Synthesis: *Bombyx mori* silk cocoons were degummed in a boiling aqueous sodium carbonate solution and then dissolved in an aqueous lithium bromide solution. The solution was dialyzed, lyophilized(7,8) and then rehydrated. The rehydrated silk solution was lyophilized a second time. The silk was then dissolved in 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP) and poured over NaCl crystals. The silk/HFIP was allowed to seep between the crystal and then the HFIP was removed. The silk was treated with methanol and then the NaCl crystals were leached.

Silk Mineralization: The porous silk scaffolds were placed in acrylic chambers attached to a peristaltic pump. Using the pump the scaffolds were treated with aqueous solutions of calcium chloride and disodium phosphate for 30 minutes each at 200mM and 100mM respectively.

Silk Characterization: The resultant silk scaffolds were analyzed with Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy to confirm mineralization.

In Vitro Study: The porous silk scaffolds and a mineralized collagen scaffold were used as controls. The experimental groups were mineralized silk and mineralized silk supplemented with either 15 or 75 µg/ml of calcitriol in the media. Human bone marrow derived stem cells were seeded at 150K cells/scaffold. Samples were analyzed at 1, 4, 7, 14, and 21 days with regular media changes over time. The media was collected for analysis of calcium and alkaline phosphatase. dsDNA was measured via PicoGreen assay and osteogenic expression (RUNX2, osteonectin and osteopontin) was assessed with polymerase chain reaction.

Statistical Analysis: Data was analyzed with a two-way ANOVA (group, time) and post hoc Tukey's test with $p<0.05$ being significant.

Results

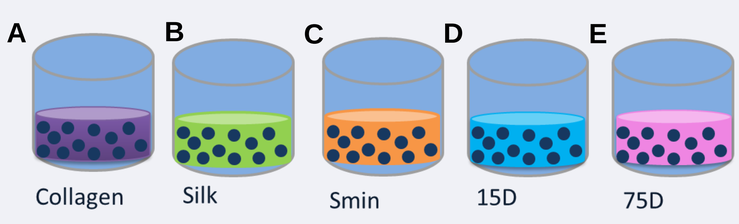
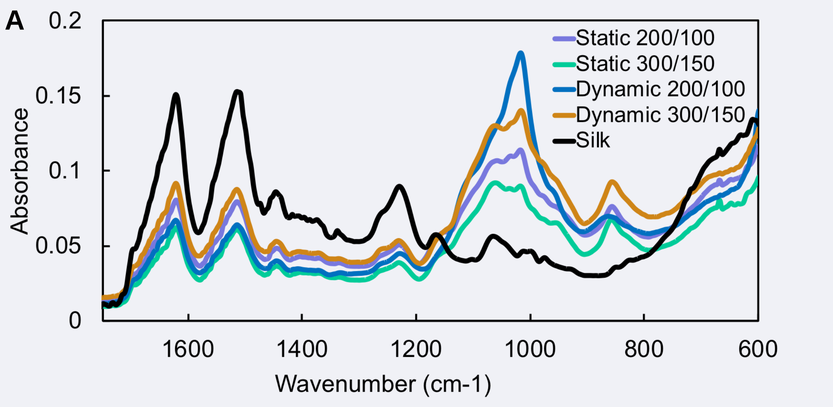


Figure 1: This figure displays the different groups used for this study: (A) Collagen scaffold, (B) Silk scaffold, (C) Mineralized silk, (D) Mineralized silk with 15 µg/ml vitamin D, and (E) Mineralized silk with 75 µg/ml vitamin D



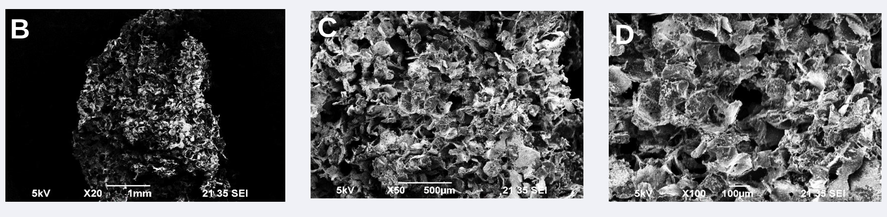


Figure 2: This figure displays the results for (A) FTIR absorbance and (B-D) SEM images for dynamic mineralization at (B) 20X, (C) 50X, and (D) 100X. The FTIR results confirm the peak shift caused by methanol treatment.

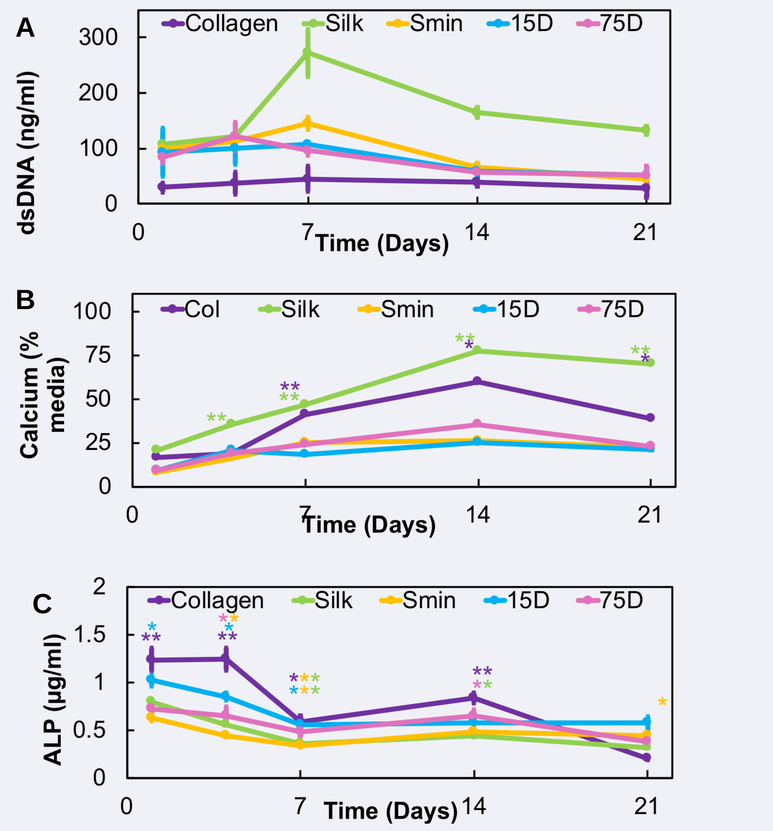


Figure 3: This figure displays the (A) calcium levels for each group at the given time points, the (B) the effects of the different treatments and scaffolds through dsDNA results, and the (C) alkaline phosphatase (ALP) activity for each group over the given time points. The statistical significance is given by different colored (*) at each time point. Two (**) of the same color indicates significance ($p<0.05$) of the group with that color compared to all other groups. One (*) indicates significance from all groups except the (**) group and asterisks of different colors show the significant between specific groups at that time point.

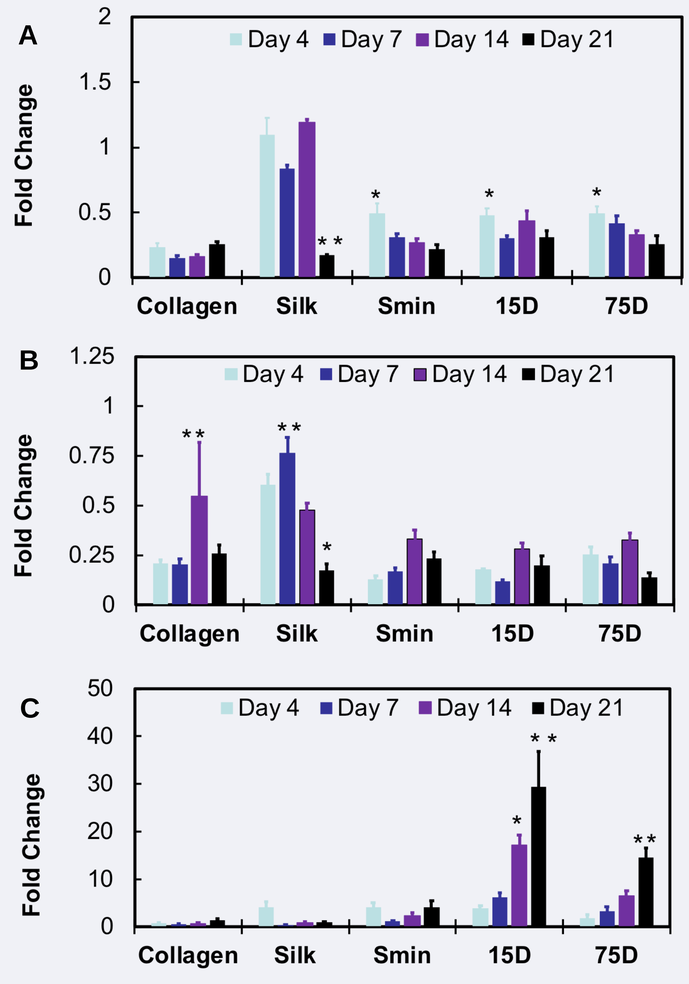


Figure 4 shows all PCR results for the expression of RUNX2 (A), osteonectine (B), and osteopontin (C) all for each group for day 4, 7, 14, and 21.

Conclusions

•Mineralization and calcitriol provide a synergistic enhancement of stem cell osteogenic expression.
•Mineralization and calcitriol maintain calcium uptake over time compared to the controls.

References

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Acknowledgments

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