Title
Effect of Silicon Ion Concentration on Osteoblast Collagen Expression

Author
Cheng, Joyce Yi-Tzy

Publication Date
2011

Peer reviewed|Thesis/dissertation

Effect of Silicon Ion Concentration on Osteoblast Collagen Expression

by

Joyce Yi-Tzy Cheng, DDS

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

GRADUATE DIVISION

of the

Acknowledgment:

I would like to thank Dr. Venu Varnasi for his guidance and efforts in the research project, Dr. Mark Ryder and Dr. Peter Loomer for serving on my committee, Benjamin Chaffee for his consistent encouragement, and all my perio faculties and co-residents who always believed I had it in me to complete the research.

Abstract:

Background: This study tested the hypothesis that various silicon ions concentrations have effects on osteoblast proliferation, gene and extracellular matrix expression. It also explored possible pathway of how silicon is effecting osteoblast differentiation.

Methods: MC3T3 cells were grow in vitro with alpha-mem solution and were treated with 10, 100, 1000uM silicon solution, and combination of Sp7/Osterix SiRNA and silicon solution. Cells were treated with cell proliferation assay, stained with Picosirius staining, and gene expressions were measured with reverse transcriptase polymerase chain reaction.

Results: Silicon treatment alone did not have an effect on cell proliferation compared to control during a 6-day period. Histology slides showed 100uM silicon has the most collagen fibrils compared to 10uM, 1000uM, and control on day 1. By day 7 the control and 100uM showed comparable amount of collagen fibrils where 10uM and 1000uM treatments had much fewer visible fibrils. Gene expression results agreed with histology slides. On day 1, cells with 100uM silicon treatment had a significant increase ($p<0.05$) in Col1α1 expression, and cells with 1000uM silicon had an increase ($p<0.05$) in Col1α2 expression. There were no significant differences in gene expression with various treatments by day 7. 100uM silicon caused significant increase ($p<0.05$) gene expressions for Sp7, Col5α3, BMP1, and Smad 5. Cells treated Sp7/Osterix SiRNA showed a significant decrease gene expression in Sp7, BMP1, Smad4, Smad5, and AKP2.

Conclusion: Silicon treatment did not effect cell proliferation, but did increase collagen expression in osteoblast cells. 100uM was the most optimum concentration for collagen expression compared to 10uM and 1000uM. One possible mechanism of how silicon

effects osteoblast cells is through BMP1, that increase Smad5 production, and leads to cells signaling for more collagen production. More work is needed to confirm the mechanistic effect of silicon.

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Introduction:

Implant dentistry has been on the rise ever since it was made popular by Brånemark in late 70s. The success rate of implants in the maxilla and the mandible are in the range of 85-100% (1). There has been tremendous interest in bone formation and repair around implants and tooth. Implant companies have been coming up with different methods to increase the rate of bone formation around their implants. Methods include surface treatments such as calcium and phosphorous coating and sandblasted and acid etched implant surfaces are advertised to promote bone to implant contact.

Products such as GEM 21, Emdogain, hydroxy apatite and Commercial Bioglass^{TM} all have been advertised to promote bone formation. While GEM 21 and Emdogain are made with organic materials, hydroxy apatite and bioglass are composed of only inorganic materials. Varansi et al showed that the ions leached from Commercial Bioglass^{TM} caused an increase in collagen type 1 production in osteoblast-like MC3T3 E1 subclone 4 (2). Collagen type 1 is often used as a marker for osteogenesis because it constitutes 90% of the total organic extracelluar matrix in mature bone, and it is synthesized by preosteoblasts or early undifferentiated osteoblast-like cells(3).

Ions from bioactive glass include Si^{4+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, and PO_4^{3-}. All the ions are present in normal growth media (α mem), but there is an increase concentration of Si^{4+} and Ca^{4+} in the bioactive glass (2). Carlisle (4) and Schwarz and Milne (5) showed the that lack of dietary silicon in chicken and rats lead to abnormally shaped bone and defective cartilaginous tissue. Reffitt (6) showed fasting Si concentration ranged from 2-10uM in human and increased to 20-30uM after meal. Reffitt looked at orthosilic

acid in vitro and saw an increased collagen type 1 production at 10-50 uM concentration(7).

A closer examination of up-stream osteogenesis regulators is needed to further understand how silicon effects collagen expression. There are multiple upstream regulators of collagen including Runx2, Wnt, hedgehog, and Sp7/Osterix. Varanasi reported there was no increase in Runx2 gene expression when osteoblast cells were exposed to the ions leached from Bioglass^{TM}. Nakashima reported without Osterix there was no bone formation, and Osterix acted down stream of Runx2(8). Feng reported that Sp7/Osterix up-regulated the mouse collagen expression in osteoblast differentiation (9). Sp7/Osterix plays an implant role of osteogenesis, and might be the pathway of how silicon effects collagen expression.

The aims of this study are to examine the effect silicon ions in vitro have on gene and extracellular matrix expression of osteogenic biomarkers; determine whether the amount of silicon ions have an effect on osteoblast differentiation; and to explore possible mechanism that caused the enhanced osteoblast differentiation.

Materials and Methods:

Study Design

Three experiments were designed to ascertain the effect of the silicon ions on MC3T3 cells in vitro. First, proliferation experiment was conducted to determine the effect of silicon ion on osteoblast growth. Second, *in vitro* differentiation experiment was conducted to determine the effect of varying ion concentrations on the various markers of osteoblast differentiation. Gene and extracellular matrix expression of these markers were

assayed in this work. Third, a gene knock out experiment was done to elucidate the possible mechanistic effect of the silicon ion. OSX gene was knocked out using siRNA kit from Santa Cruz Biotechnology and the genetic markers of osteoblast differentiation were assayed.

Treatment medium preparation

The basal medium was prepared by supplementing α-MEM, with 10% FBS and 1% pen-strep. To make treatment media of varying silicon ion concentrations, sodium metasilicate, Na_2SiO_3 (Aldrich #307815) was used for silicon ion additions to α-MEM. 10000 uM Si concentration was made, and was serial diluted to 1000 uM, 100 uM, and 10 uM.

Treatment media for the proliferation and the differentiation experiment were adjusted to pH of 7.5 from 8.0 by adding TRIS-HCl buffer. No Tris buffer was added in the mechanism study.

In vitro osteoblast proliferation experiment

Prior to all experiments, MC3T3-E1 sub clone 4 cells were cultured in 150 cm^2 flasks. Cells were seeded at a density of 5,000 cells cm^{-2}.

MC3T3-E1 cells were cultured in a 96 well plate. The basal medium was replaced with 1000 uM, 100 uM, and 10 uM silicon addition, and basal media (control) treatments one hour post seeding. No ascorbic acid or glycerol 2-phosphate was used in

the proliferation experiment. Cell cultures for each treatment were conducted in triplicate, and were cultured for 0.16,1, 2, 3, 4, 5 and 6 days.

At the desired time point, each well plate was removed from incubation and assayed for viable cell numbers. All treatments were exchanged with the cell proliferation assay treatment (α-MEM, 10% FBS, 1% pen-strep, 317 μg ml^{-1} MTS reagent as described by the manufacturer's protocol) and were incubated for an additional for 4h. The color of the formazen product released by the cells was then analyzed using a spectrophotometer (490 nm, SpectraMax Plus, Molecular Devices, San Jose, CA) to quantify cell numbers relative to standard curves.

The MTS One Solution Assay (Promega, Madison, WI) is a colorimetric assay, which is used for measuring the metabolic activity of viable cells during proliferation. The assay reagent contains a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium,innersalt;MTS] and an electron coupling reagent (phenazine ethosulfate; PES). These reagents are combined into one solution, in which the tetrazolium product is bioreduced by cells into a formazen product, which colors the solution and indicates the numbers of viable cells. This assay has shown similar sensitivity to viable cell metabolic activity compared with the MTT assay(10). Standard curves for the use of this assay kit for this cell line were used to determine cell numbers.

In vitro differentiation Experiment

MC3T3-E1 sub clone 4 cells were cultured in 150 cm^2 flasks (passage 25–30) prior to seeding (50,000 cells/well) in 6-well tissue culture plastic plates and incubated overnight. After the cell line doubling time (12–16 h), these cells were synchronized (α-MEM, 1% FBS, 1% pen-strep) for an additional 48 h. Synchronization media was replaced with silicon ion treatments and basal media with additional supplementation of ascorbic acid (50 ppm) and β-glycerophosphate (50 pmm). Basal media served as control. The following table represents the treatments laid out in 6 well plates:

1000uM Si	1000uM Si	1000uM Si
100uM Si	100uM Si	100uM Si

10uM Si	10uM Si	10uM Si

Control	Control	Control
Control+tris	Control+tris	Control+tris

Reverse transcriptase polymerase chain reaction

To quantify level of gene expression, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was used. Cells were lysed and RNA was recovered using RNEasy Mini Kit (Qiagen, Valencia, CA). Extracts were converted to

cDNA using reverse transcriptase (Reverse Transcription System, Promega, Madison WI) according to manufacturer's protocol. Asorbance (A) measurement of mRNA and cDNA samples were performed using a full spectrum UV/Vis nanodrop volume analyzer (ND-1000, Nanodrop Technologies, Wilmington, Delaware). Concentrations of total RNA or total cDNA were measured at 260 nm (A_{260}). The measurement of nucleic absorbance A_{260}/ A_{280} is commonly used to assess the purity of nucleic acids after retrieval from cell and tissue cultures. All cDNA samples were diluted to 100ng/ul.

Samples were mixed with reagents for PCR as follows (final concentration): cDNA samples (10%), FastStart Taqman Master Mix (Rox, 2x, 50%) (Roche Applied Sciences, Mannheim, Germany), forward primer (900nM), reverse primer (900nM), and hydrolysis probe (250nM) mixture (seen in table 1) and PCR grade water (Amgen Inc, South San Francisco, CA). Sample reaction was performed using a real time PCR machine (ABI7500, Applied Biosystems inc CA).

Table 1

Gene	**Accession No.**	**Gene bank mRNAs**	**Amplicon length**
GAPDH	NM_008084.2	31	107
ALP1	NM_025610.3	4	66
Lox	NM_010728.2	15	73
RUNX2	NM_009820.2	2	115

Gene	Accession No.	Gene bank mRNAs	Amplicon length
COL1α1	NM_007742.3	8	89
COL1α2	NM_00773.2	18	140
Bglap-rs1	NM_031368.4	10	110

All amplifications were compared to an internal housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase or GAPDH) for relative quantification. Data acquisition was performed using the Applied Biosystems 7500 thermal cycler software package. Statistical analysis was conducted on amplification curves using Sigma Plot software package (Systat Software Inc., San Jose). Statistical analysis on the amplification curves was performed using sigmoidal curve fitting (following the procedure outlined by Qui *et al*). Regression of these sigmoidal fits were performed using a 4-paramter model offered by SigmaPlot software package. A strengthen of non-linear fit (R^2) and the presence of an adequate plateau of the amplification curve were used as criteria for which curves were evaluated for their threshold cycle (C_T). Amplification curves having an R^2 less than 0.95 were not used.

Relative comparison of C_T values was calculated as long was the expression of GAPDH gene did not change as a function of time or treatment. Statistical analysis was done using the delta-delta C_T values, the variance in the data is associated with the concentration of target cDNA relative to the concentration of the internal reference gene cDNA.

Gene expression was studied at day 7 of differentiation because this period marks the early expression of differentiation of osteoblast phenotype.

Histology

Cells were seeded (20,000 cells/well) onto glass cover slips seated in 12-well plates. Cells were treated in experimental and basal media with additional supplementation of ascorbic acid (50 ppm) and β-glycerophosphate (50 pmm). The experiment was carried out over 30 days, during which samples were removed from incubator on day 1, 2, 7, 21, and 30. Samples were transferred to a fresh well plate, washed twice in PBS and fixed using Bouin's fixative solution (Richard-Allen Scientific, Kalamazoo, MI) under ambient conditions for 1 h, with excess fixative rinsed off with deionized water. Bouin's fixative is used to enhance the binding of Picrosirius staining of collagen fibrils while using a Fastgreen background tissue stain. Cell layers were then stained with 0.1% Fastgreen (Sigma Inc.) for 30 min, with excess staining removed using glacial acetic acid (American Master Tech Scientific Inc., Lodi, CA) for 30 min. Fastgreen (light green or forest green, depending on tissue density) was used as a counter stain for Picrosirius stain, and its intensity increased with increasing extracellular matrix tissue density. Cell layers were then stained using Picrosirius stain for 1 h, with excess stain washed off using deionized water. Picrosirius stains collagen fibrils and is enhanced using polarized light owed to the birefringence of collagen. Cell layers were then sequentially alcohol dehydrated (70–100% ethanol) for 30 s to remove excess water prior to imaging. Cell layers were imaged using an optical microscope (Olympus BX51, Tokyo, Japan) with a CCD camera and Image Pro software. When imaged using

polarized light, Picrosirius stain (on a fast green counter stain) stains collagen type 1 red, orange or yellow.

Mechanism study using siRNA transfection experiments

The siRNA cocktails targeting mouse Sp7/Osterix were purchased (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). The mouse MC3T3-E1.4 osteoblastic cells were transfected according to Santa Cruz Biotechnology protocol. The only deviation from the protocol was the amount of siRNA added to each well changed from 60pmol to 120pmol because we seeded twice as many cells as the protocol suggested.

400,000 cells were seeded in each of the wells instead of 200,000 cells per well listed in the protocol. Seeded 400,000 cells/well in 6 well plates with basal media for 24 hours. The cells were then synced with 99% alpha mem solution with 1% of FBS for 48 hours. Transfection were done with siRNA cocktail according to protocol listed by Santa Cruz Biotechnology Inc. Once the transfection was completed, the wells were filled with 100uM Si or basal media as control. The wells are plated as the following:

siRNA (100 uM Si)	siRNA (100 uM Si)	siRNA (100 uM Si)
siRNA (regular media)	siRNA (Regular media)	siRNA (Regular media)

No siRNA 100uM Si	No siRNA 100uM Si	No siRNA 100uM Si
No SiRNA	No SiRNA	No SiRNA

Regular Media	Regular Media	Regular Media

Control siRNA (100 uM Si)
Control siRNA (regular media)

The control siRNA is used as a negative control for experiments using targeting siRNA transfection; each consist of a scrambled sequence that will not lead to specific degradation of any known cellular mRNA.

72 hours after the treatment was introduced the cells were lysed, converted into cDNA, and prepared for quantitative PCR in the same fashion as previously described. In table 2 there is the list of gene assay that is used for the quantitative PCR for the mechanism study.

The study was repeated twice and only the gene expression that showed consisted trend between the repeated experiment were reported in the paper.

Table 2

Gene	**Accession No.**	**Gene bank mRNAs**	**Amplicon length**
GAPDH	NM_008084.2	31	107
ALP1	NM_025610.3	4	66

Gene	Accession No.	Gene bank mRNAs	Amplicon length
Lox	NM_010728.2	15	73
RUNX2	NM_009820.2	2	115
COL1α1	NM_007742.3	8	89
AKP2	NM_00773.2	18	140
Bglap-rs1	NM_031368.4	10	110
Sp7	NM_130458.3	3	88
Col5a3	NM_016919.2	5	106
Col3a1	NM_009930.2	19	136
Smad1	NM_008539.3	7	63
Smad5	3 ref sequence	5	159
Bmp1	2 ref sequence	6	52
Bmp2	NM_007553.2	4	58
Col5a1	NM_015734.2	3	125
Smad4	NM_008540.2	9	71

Statistics

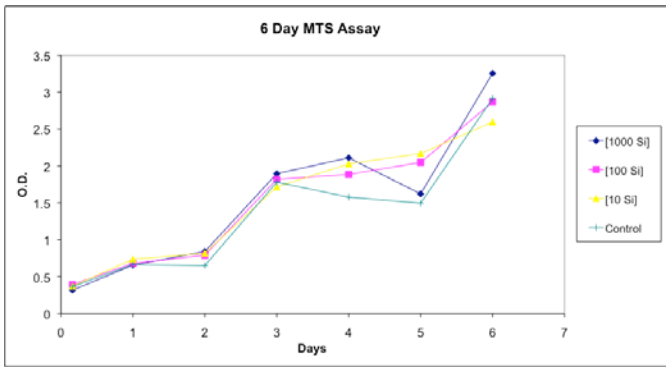
Data analysis was conducted to determine the statistical significance ($p < 0.05$). Two-way and one-way analysis of variance (ANOVA) testing was used to compare the treatments of various concentrations of Si ion addition and control on MC3T3-E1 sub clone 4-cell proliferation. For gene-related studies, genes expressed as a result of exposure of cells to different treatments were reported relative to the control treatment (100%). All between-group comparisons were made using Tukey's analysis. Comparisons between α-MEM, and various ion concentrations were analyzed for statistical significance using one-way ANOVA with Tukey's analysis for between-group comparisons.

Results:

Effect of silicon ion treatment on proliferation of osteoblast cells:

1000uM, 100uM, 10uM silicon ion did not a significant effect on proliferation of MC3TC cells compared to the control. MC3TC cells increased in number steadily from day 1 to day 6 regardless of the type of media as seen in figure 1. Silicon ion treatment did not have a negative or a positive effect on cell proliferation compared to control.

Figure 1: Proliferation of MT3TC cells with various Si ion concentrations



Effects of Si ions on osteoblast differentiation:

Histology:

MC3TC cells were viewed under polarized microscope at 200x at day 1 and day 7 after treatments were introduced. Figure 2 showed the extracellular layer stained with Picosirious on day 1. On day 1 there was greater collagen density with 100uM Si compared to 10uM, 1000uM, and control. The collagen fibril showed up as greenish yellow fibril that was orthogonal due to the birefringent nature of the Picosirious staining. At 100uM Si there were several distinct areas that showed collagen fibers versus none at 10uM, 1000uM, and control, which was pointed out by the white arrows.

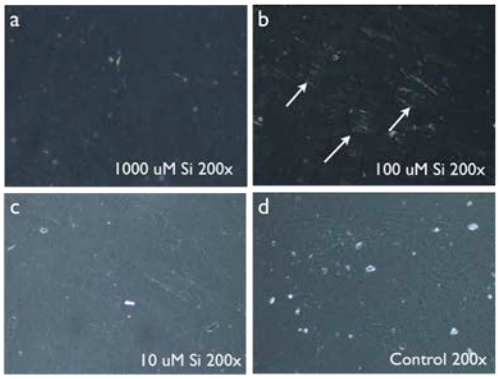


Figure 2 Optical micrograph showing extra cellular matrix as result to exposure of MC3TC-E1subclone 4 exposure to various concentration of silicon after day 1. The white arrow indicates the presence of fibrillar collagen, which is seen as the greenish yellow streaks in figure 2-b

After 7 days of treatment, there was an increase in collagen density compared to day 1. Figure 3 showed the extracellular layer stained with Picosirious on day 7 under a polarized microscope at 200x. As seen from figure 2 to figure 3, the amount of collagen has increased dramatically from day 1 to day 7. On day 7 there was similar collagen

density with 100uM Si and control. 100uM Si and control has greater amount collagen fibrils than 10uM and1000uM Si. There is little to no collagen expression in the extracellular matrix in the cells that were treated with 10uM and 1000uM Si.

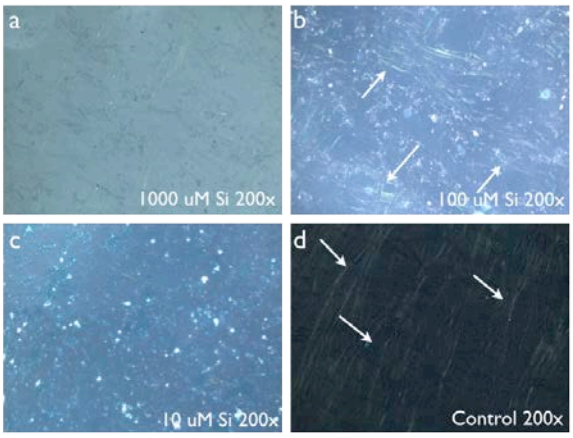


Figure 3. Optical micrograph showing extra cellular matrix as result to exposure of MC3TC-E1subclone 4 exposure to various concentration of silicon after day 7. The white arrow indicates the presence of fibrillar collagen, which is seen as the greenish yellow streaks in figure 3-b and 3-d

Reverse transcriptase polymerase chain reaction:

At day 1 and 7, gene expression was measured with reverse transcriptase polymerase chain reaction. All the gene expressions were compared to the internal reference gene GAPDH and reported as a relative expression compared to the control media. Figure 4 showed the relative expression level between all of the different treatments for col1α1 and col1α2 at day 1. Control treatment was calculated to have the relative expression of 1 since it was used as a baseline. Cells that received 100uM Si treatment showed a significant increase ($p<0.05$) in col1α1 expression, while cells received 1000uM Si treatment showed significant ($p<0.05$) increase in col1α2 expression compared to the control. There were no significant differences in relative gene

expression between treatment groups for ALP1, LOX, RUNX2, and Bglap-rs, and on day 7 there was no difference between any of the treatments.

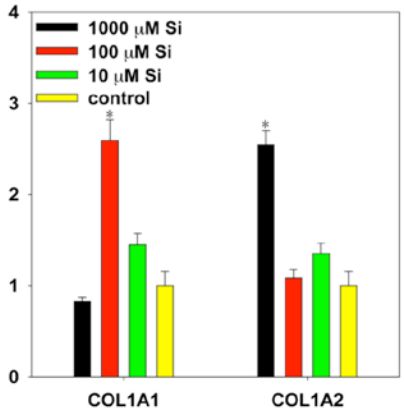


Figure 4. Relative gene expression between all treatments for col1α1 and col1α2 on day 1. 100uM treatment has the significantly more col1α1 then the rest of the treatments, while 1000uM treatment has significantly more col1α2 then the rest of the other treatments (*indicates $p<0.05$)

Mechanism study using Sp7/OSX SiRNA:

Collagen gene expression was most robust for 100uM compared to 10uM, 1000uM, and control based on results from the histology and polymerase chain reaction experiment. Sp7/Osterix SiRNA kit from Santa Cruz Biotechnology was used to knock down Sp7/Osterix in MC3TC. All the gene expressions were compared to the internal reference gene GAPDH and reported as a relative expression compared to the control media. Figure 5 showed the relative expression level for control, 100uM Si treatment, control with Sp7/Osterix SiRNA, and 100uM Si treatment with Sp7/Osterix SiRNA. The relative expression level for control was calculated to be 1.

There was no statistical difference in the relative gene expression for col1α1 and col3α1 between control, 100uM Si treatment, control plus Sp7/Osterix SiRNA, and

100uM Si treatment with Sp7/Osterix SiRNA. The Applied Biosystems 7500 thermal cycler software was not able to detect consistent amplification for ALP1, Runx2, Bglap-rs, Lox, col5α1, Smad1, and BMP2.

Sp7 has a significant increase ($p<0.05$) in gene expression when it was treated with 100uM Si compared to control, and it significantly ($p<0.05$) decreased in treatments with Sp7/Osterix SiRNA. Bmp1, col5α3, and Smad5 showed a similar trend with Sp7 with an increased expression in 10uM Si compared to control and decreased expression with treatments with Sp7/Osterix SiRNA. Smad5 showed the most increase ($p<0.05$), more than 8 folds, in gene expression when it was treated with 100uM Si. Silicon treatment and cells treated with Sp7/Osterix SiRNA significantly ($p<0.05$) suppressed Smad4 expression. AKP2 expression was suppressed significantly ($p<0.05$) when the cells were treated with Sp7/Osterix SiRNA.

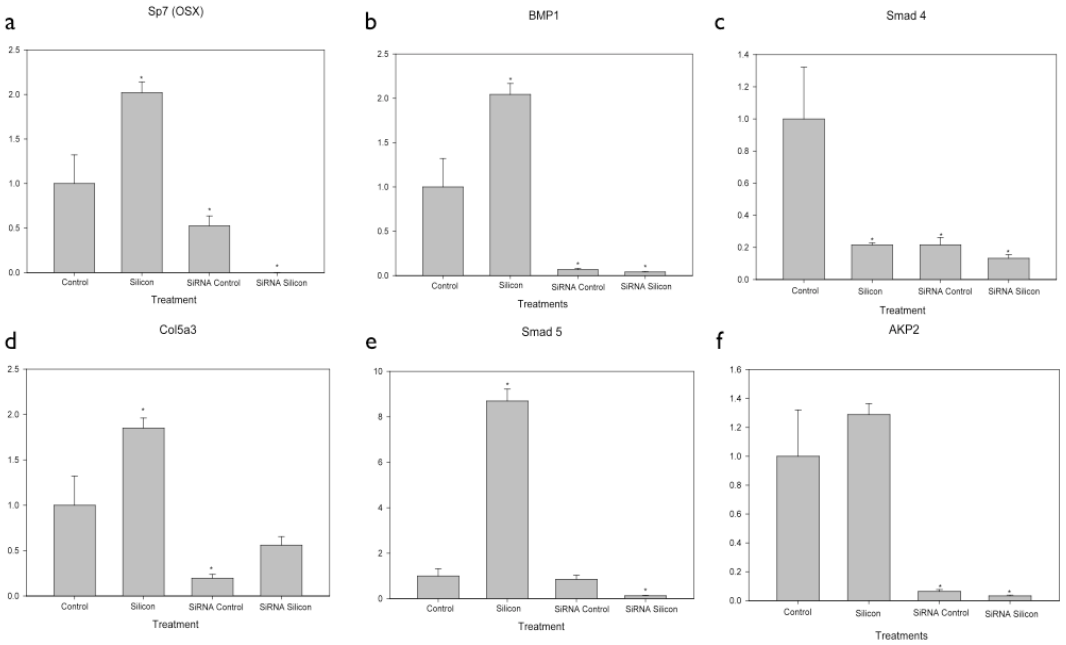


Figure 5. Relative gene expression for MC3TC cells treated with control media, 100uM Si, control media with Sp7/Osterix SiRNA, 100uM Si with Sp7/Osterix. * indicates $p<0.05$. Sp7, BMP1, col5a5, and Smad 5 showed similar trends with increase in expression with 10uM Si, while Smad4 had a decrease in gene expression with 10uM Si treatment.

Discussion:

There was no significant difference in the cell proliferation pattern between treatments. Although at on days 3 and 4 the silicon treatments have higher cell density compared to control by day 6 there wasn't a distinguishable difference. The results indicated that although there was slight initial increase in cell proliferation, by day 6 the silicon treatment did not have any advantage over the control. A Pubmed search was done to compare this result to other labs, but there was no report of effect of silicon solution on the proliferation of MC3T3 cells. Shie et al looked at MG63, which is human osteoblast cell, and saw there was an increase in cell proliferation in silicon concentration of 2 and 4 mM, but no increase in cell growth with control from day 1-7 (11). The discrepancy between our studies might be due to the type of cell that was used, the type of media, and Shie was using a much higher concentration of silicon than what was used in our experiment.

The histology supports the result obtained from the RT-PCR results for the cell differentiation experiment. From figure 4, 100uM Si showed the highest col1α1 gene expression and it was confirmed visually in the histology slide shown in figure 2. The result also correlates with the previous work by Varnasi et al, where they showed that at day 1 there was the most increase in col1α1 gene expression in MC3T3 cells in ion solutions containing silicon ions.

100uM gave the most optimal result for increase in collagen expression out of control and the two other silicon concentrations, and it was the concentration that was chosen to test the whether silicon acted through Sp7/Osterix to increase collagen expression.

Kazuhisa et al had identified Sp7/Osterix (Osx) as zinc containing transcription factor that was expressed in osteoblasts in endoconchondral and membranous bones, and in Osx null mice there was no endochondral nor intramembraneous bone formation. They also found that Osx worked down stream of Runx2/Cbfa1 in osteoblast differentiation(8). In our experiment, Sp7/Osx gene expression increased significantly ($p<0.05$) in the presence of silicon treatment, while there was no effect on Runx2 gene expression. Our result indicated that silicon ion's effect on osteoblast differentiation occurred down stream of Runx2, but before or at Sp7/Osx.

The purpose of the SiRNA kit was to reduce the amount of Sp7/Osx gene, and our results showed the kit was valid in figure 5a. Silicon treatment increased the expression on col1α1 on day 1, but had no effect on col1α1 expression by day 3 or 7 although there was on increase in col5α3 on day 3. On figure 5d, it showed that col5α3 expression increased significantly with silicon treatment on day 3, and even with Sp7/Osx SiRNA it had a higher expression with silicon treatment than in control with Sp7/Osx SiRNA. The result agreed with the results from Yun-Fen et al, where they showed that Sp7/Osterix up-regulates col5α3 during osteoblast differentiation (9). Col5α3 is expressed in bone and influences bone formation by osteoblasts through proteoglycan on the cell surface(12). Silicon treatment caused

an increase in Col1α1 expression but was not able to sustain the increase by the day 3, but its effect on collagen expression was maintained through col5α3.

BMP1, Smad4, Smad5, and AKP2 all worked down stream of Sp7/Osx since there was a consistent decrease in gene expression regardless of the treatment. AKP2 was not effected by silicon treatment. BMP1 and Smad 5 showed a significant increase ($p<0.05$) in gene expression with silicon treatment, while Smad4 was down regulated ($p<0.05$).

One possible explanation of increase in collagen expression with silicon treatment is that silicon up-regulates BMP1. BMP1 belongs to the astacin family and is known to have a regulatory function for extracellular matrix (ECM) formation through processing the c-propeptides of pro-collagens, and has been speculated to orchestrate ECM formation by means of signaling by TGFβ like proteins(13). Grgurevic et al recently showed that BMP1 increased bone repair in rabbits and increase collagen type 1 production in MC3T3 cells (14). TGFβ family communicates to target genes by activating Smad1, 3, 5, and 8(15). Retting el al showed that Smad1 and Smad5 are required to show effect of BMP, which belongs to TGFβ family, in endochondral bone formation(16). Smad4 does not get activated by the TGFβ family(15), and thus wasn't effected positively by the silicon treatment.

Conclusion:

Silicon ion did not effect the proliferation of MC3TC cells. 100uM silicon showed most collagen production compared to 1000uM and 10uM silicon. 100uM

silicon ion treatment had induced significant increase in Col1α1 gene expression on day 1 and an increase in Col5α3 on day 3. A possible mechanism of action is silicon is up regulation of BMP1 expression, which in turns activates proteins in TGFβ family. TGFβ family then uses Smad protein to form transcriptional complexes to target collagen genes. More work needs to be done to fully elucidate the effect silicon ions on osteoblast cells.

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Tables:

Table 1

Gene	Accession No.	Gene bank mRNAs	Amplicon length
GAPDH	NM_008084.2	31	107
ALP1	NM_025610.3	4	66
Lox	NM_010728.2	15	73
RUNX2	NM_009820.2	2	115
COL1α1	NM_007742.3	8	89
COL1α2	NM_00773.2	18	140
Bglap-rs1	NM_031368.4	10	110

Table 2:

Gene	Accession No.	Gene bank mRNAs	Amplicon length
GAPDH	NM_008084.2	31	107
ALP1	NM_025610.3	4	66
Lox	NM_010728.2	15	73
RUNX2	NM_009820.2	2	115

Gene	Accession No.	Gene bank mRNAs	Amplicon length
COL1α1	NM_007742.3	8	89
AKP2	NM_00773.2	18	140
Bglap-rs1	NM_031368.4	10	110
Sp7	NM_130458.3	3	88
Col5a3	NM_016919.2	5	106
Col3a1	NM_009930.2	19	136
Smad1	NM_008539.3	7	63
Smad5	3 ref sequence	5	159
Bmp1	2 ref sequence	6	52
Bmp2	NM_007553.2	4	58
Col5a1	NM_015734.2	3	125
Smad4	NM_008540.2	9	71

Figures

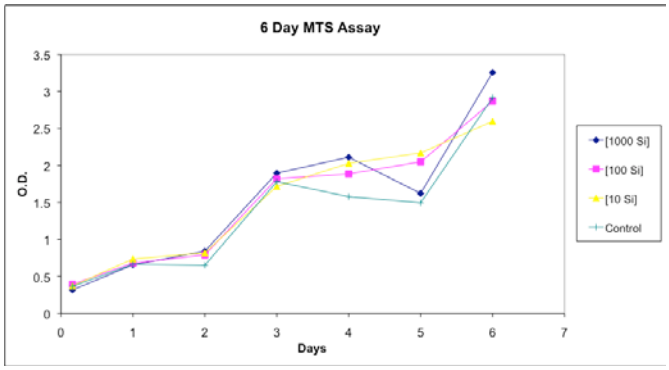

Figure 1: Proliferation of MT3TC cells with various Si ion concentrations

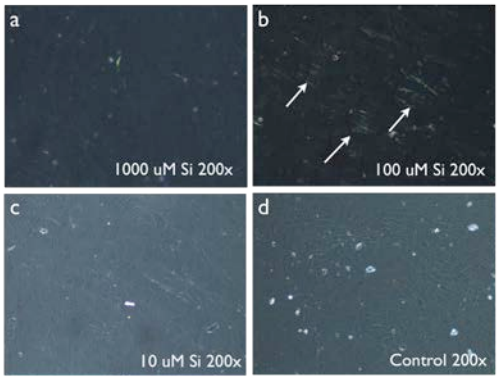

Figure 2: Optical micrograph showing extra cellular matrix as result to exposure of MC3TC-E1subclone 4 exposure to various concentration of silicon after day 1.

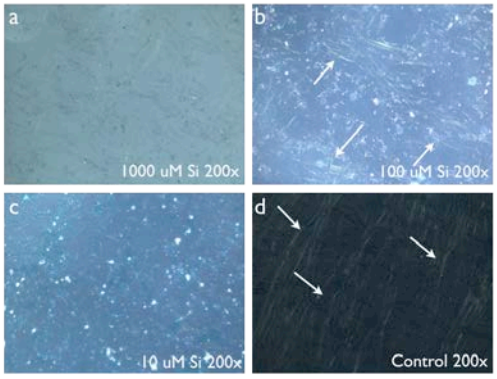

Figure 3: Optical micrograph showing extra cellular matrix as result to exposure of MC3TC-E1subclone 4 exposure to various concentration of silicon after day 7.

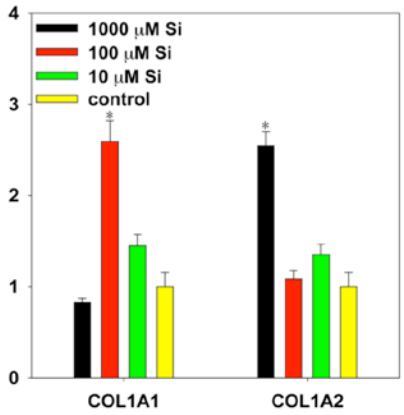

Figure 4: Relative gene expression between all treatments for col1α1 and col1α2 on day 1.

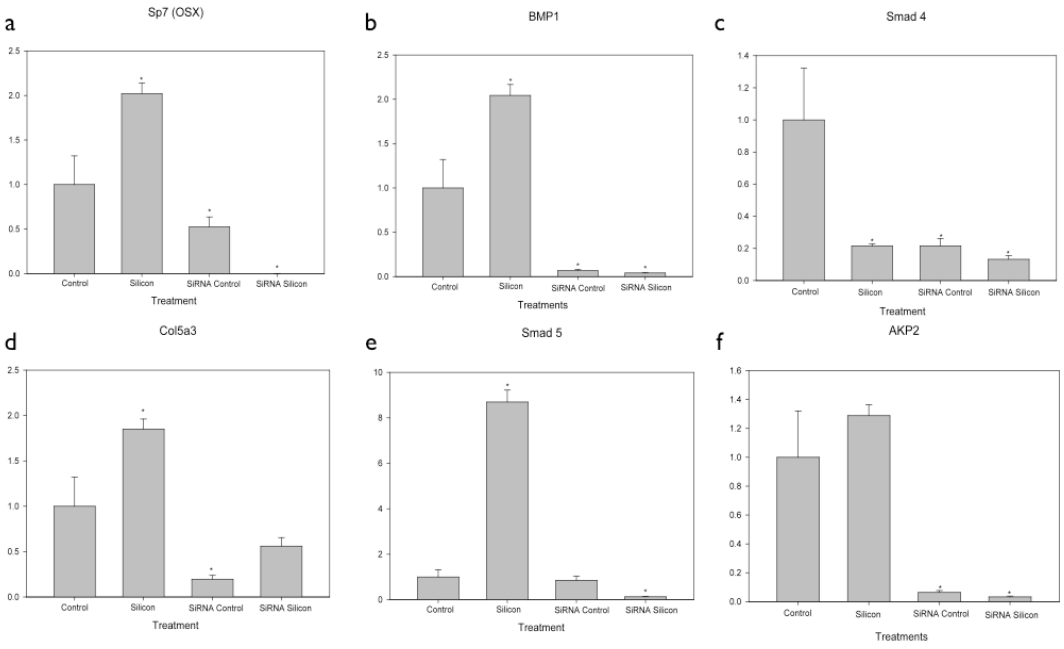

Figure 5. Relative gene expression for MC3TC cells treated with control media, 100uM Si, control media with Sp7/Osterix SiRNA, 100uM Si with Sp7/Osterix.

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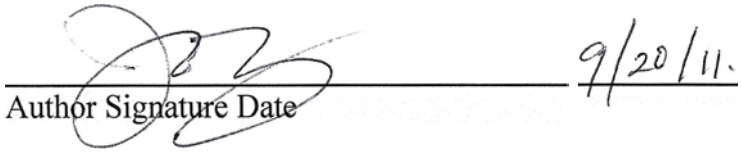

Author Signature Date 9/20/11