Zinc Loaded Calcium Silicate Particles Positively Regulate Runx2 Expression by Targeting miR-3148 in Human Osteoblastic Cells

Muskan Soni

Muskan Medical Assistance Centre, Orissa, India

Corresponding author: [starlike6987@gmail.com](mailto:starlike6987@gmail.com)

**Abstract:** Introduction: Runx2 gene (known as Runt-related transcription factor 2) plays an important role for bone formation as well as osteoblast differentiation. It controls many osteogenesis genes, collagen production and mineralization. The developmental steps involved in transforming pre-osteoblast into mature, fully functional osteoblast is known as ‘Osteoblast differentiation’. Osteoblastic differentiation is under tight control of multi-signaling and multi-transcription routes. The potential for calcium silicate particles on Runx2 gene lies in their ability to influence the osteogenesis and formation of bone tissue. They are biocompatible and bioactive providing interaction between the surrounding cells and tissues of the body. Upon contact with osteoblasts, osteogenic differentiation may occur leading to the formation of osteoclasts. Calcium silicate particles stimulate Runx2 production or gene expression. Materials and methods: List of miRNAs targeting Runx2 were obtained from mirDB database. Data obtained from Targetscan Predicted SEED region (paring region) by miR-3148 in Runx2 UTR. After which Runx2 expression in osteoblasts treated with zinc-doped calcium silicate particles (Zn-Cs) for 3d under osteogenic medium were checked. Discussion and conclusion: After treating osteoblasts with zinc-doped calcium silicate particles, it enhanced their RUNx2 expression under osteogenic conditions (cell culture, medium along with vitamin C, beta-glycerol, phosphate, and dexamethasone) by downregulating microRNA, which targets RUNx2. Therefore, promoting bone formation. Zinc-doped calcium silicate particles are generally used for bone tissue regeneration purposes. Now we have found one such molecular mechanism behind this osteogenic formation. So from this, we can interpret similar mechanisms that could regulate RunX2 In our futuristic studies.

# INTRODUCTION

The essential need for bone tissue regeneration is driven by the rise in bone losses, critical-sized bone defects, delayed bone unions and maxillo-facial surgery. Even with surgical intervention, bone injuries frequently mend poorly, and treating them has remained a serious clinical problem with considerable effects on patient quality of life and medical costs across the board. Bone resorption and bone defects are currently treated with bone grafts taken from the patient's iliac crest or ribs (autografts) or from other donors (allografts), which are currently the majority of methods for treating and preventing osteoporosis that are currently available[(Ganapathy 2003)](https://paperpile.com/c/DBJKSf/jFOS+xB9P). These methods involve medications that suppress osteoclast activity. Nevertheless, despite significant advancements in this area, the presently available substitutes of bone replacements still have a weak capacity to endure skeletal stresses or to actively promote bone growth[(Jadlowiec et al., 2003; Meenakshi & Sankari, 2021)](https://paperpile.com/c/DBJKSf/jFOS+B96y).

Metals, polymers, ceramics, and their composites are the primary materials used in synthetic bone substitutes.[(Chokkattu et al., 2022; Merchant et al., 2022)](https://paperpile.com/c/DBJKSf/ttka+Atsd) The most recent biomaterials are bioactive and biodegradable substances that are designed to encourage specific cellular reactions at the molecular level that enhance tissue regeneration. They are successfully used in orthopedic and dentistry applications. [(Pandiyan et al., 2022)](https://paperpile.com/c/DBJKSf/oO4m)

The basic idea of perfect bone substitutes is that resorption can gradually renew the body's own biological tissues while the mechanical qualities must be tailored to human bone. Currently, calcium silicate-based ceramics are being investigated as a possible replacement for calcium phosphate ceramics. Ceramics made of calcium silicate have a propensity to release ions at concentrations that encourage osteoblast development and proliferation. Ca plays a crucial function in the development of blood vessels and bone by being present in the active area of natural bone. Calcium concentrations between 2-4 mmol favored osteoblast development and proliferation, while between 6-8 mmol favored extracellular matrix mineralization. Therefore calcium can be used in bioceramics. [(Padmanabhan et al., 2022)](https://paperpile.com/c/DBJKSf/gCyK).

Silica has a beneficial effect in bone health and bone production, and studies have suggested that silicon may have a role in bone tissues. Si is beneficial for increasing bone density and preventing osteoporosis since it is necessary for the metabolic process involved in bone calcification. According to certain reports, Si can also promote osteoblast development and proliferation while controlling the activation of genes relevant to bone[(Arvind et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/DBJKSf/Y4N6+CDU6).

Furthermore, biological performance can be improved by doping it with other elements such as strontium (Sr), silicon (Si), magnesium (Mg), and zinc (Zn), which are constituents of human bone in varying proportions depending on the type of tissue (i.e., bone, dentin, enamel). These doped elements have a crucial role in bone repair and biomineralization [(D. Hu et al., 2016; Marya et al., 2022)](https://paperpile.com/c/DBJKSf/cVo7+OIih).

Zinc is a structural component of several proteins, such as transcription factors and enzymes involved in cellular signaling pathways, which promote the proliferation, differentiation, and mineralization of osteoblastic cells. The prevention of osteoporosis may benefit from zinc supplementation. [(Jain & Verma, 2022; Wadhwani et al., 2022)](https://paperpile.com/c/DBJKSf/onfi+zpme) According to certain research, Zn, Mg, and Sr ion implants increased osteoblastic cell proliferation and improved osseointegration in bone, whether it was healthy or pathological.tests revealed that Zn-modified calcium silicate coatings had a significant influence on the new bone formation. The effects of the ion-doped bioactive coatings on bone-forming cells are scientifically promising, but the molecular mechanism behind these benefits is not entirely known[(Yuan et al., 2023)](https://paperpile.com/c/DBJKSf/eG92).

Runx2, a transcription factor that plays an important role in osteogenesis and bone formation processes, is considered as one of the key players in these processes. RUNx2 acts as a target for zinc-calcium silicate particles for their bone tissue-forming benefits. Differentiating mesenchymal stem cells into osteoblasts may be aided by regulating the level/function of RUNX2 mRNA which is a bone regeneration factor. [(Sreevarun et al., 2023)](https://paperpile.com/c/DBJKSf/4RKx) They are comprised of short RNAs called miRNAs that have a length of about 22 nucleotides which bind the complementary portions of the untranslated regions (UTR) of mRNAs thereby triggering either degradation or translation arrest. Firstly, these are translational products of diverse functions which include essential roles in several biological activities. A number of genetic pathways related with the differentiation of progenitor cells into osteoblasts and osteocytes were studied within the framework of a cell experiment aimed at determining the potential roles of miRs in osteogenesis. Osteoblast differentiation undergoes various signaling pathways such as TFG and BMP regulated by miRNA during skeletal development. During skeletal formation, runx2, osx, and atf4 transcription factors guide the development of osteoblast precursors. Some of these nuclear proteins include large numbers that either inhibit or enhance the regulation of the factors themselves[(“MicroRNA Function in Craniofacial Bone Formation, Regeneration and Repair,” 2021)](https://paperpile.com/c/DBJKSf/w0o4).[(Adel et al., 2023; Subramaniam et al., 2023)](https://paperpile.com/c/DBJKSf/AKRo+NCBV)

Several biomaterials that have been documented as exhibiting osteo-integrative, osteoconductive, and osteoinductive features towards bone tissue engineering are also among some of these materials. Investigating the expression of miRNAs and material-regulated-miRNAs will enhance the practical aspects of using these biomaterials for bone growth. Bioinformatics tools and offer vasts of data regarding different bone-related MiRNAs. [(Solanki et al., 2023; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/DBJKSf/8Yvx+Srk9)These include important miRNAs for regulation of osteogenesis by a biomaterial, which will provide an estimation for the osteogenic value of the biomaterial. For instance, the regulation of miR-30c by nano-bioglass ceramic (nBGC) is one good example of work. miR-30c expression was highly expressed which has Tgf2 and Hdac4 identified as the targets of the gene.These two genes inhibit bone formation. By upregulation of miR-30c. They also improve bone formation by turning off their target genes[(“Biomaterials Mediated microRNA Delivery for Bone Tissue Engineering,” 2015)](https://paperpile.com/c/DBJKSf/wdv1)[(Sugumaran et al., 2023; Wang et al., 2022)](https://paperpile.com/c/DBJKSf/lFuW+1quE).

MiRNAs expression profile associated with osteogenic differentiation media may vary depending on cell culture type compared to in vivo bone development. [(Chokkattu et al., 2023; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/DBJKSf/AZn8+1IcB)Osteogenic media can alter any number of different cells into expressing miRNAs and in the same manner multiple miRs can trigger a bone forming state within cells under an osteogenic condition. This strategy has the potential to lead to the creation of more potent treatments for skeletal illnesses like fractures and bone deformities. So the aim is to study the Runx2-Targeting miRNAs Regulated by Zinc-Loaded Calcium Silicate Particles[(Yuan et al., 2023)](https://paperpile.com/c/DBJKSf/eG92).

# MATERIAL AND METHODS

## CELL CULTURE

The normal hone osteoblastic cells designated as MG-63 obtained from NCCS (National Centre of cell Science), Pune, was cultures under routine circumstances.First, cells were cultured and a series of cell culture experiment followed. In experiments designed to assess gene expression of osteogenic differentiation marker genes a medium of osteogenic induction (normal medium having addition of beta-glycerophosphate, dexamethasone, and vitamin-C) was combined with normal culture condition.

Vegetable waste that was discarded was obtained locally and burnt at 500 ° C for three hours until the conversion of sodium silicate to white ash. After the conversion, sodium silicate underwent processing together with one molar calcium nitrate solution to acquire This white calcium silicate ppt was subsequently stirred for four hours and filtered as well, followed by washing of deionized water. Finally, the precipitate was washed twice with absolute ethanol. Powder drying at 80°C for 24 hours.

HCI solution was used in order to create the mesoporous characteristic of wollastonite. A slurry of dry calcium silicate powder emulsified in deionised water. The addition of 1N HCL adjusted this slurry at Ph 4, 5 & 6 and the same was eventually dried. After this, the product was categorized as CS-4. 0, CS-5. 0, and CS- 6. 0.

Then 0.1 mm of zinc chloride solution was added to 1 gm of particles(calcium silicate) dipped and stirred for 24 hours. Later the particles were washed and air dried giving us zinc loaded calcium silicate particles.

## Biocompatibility assessment using MTT assay

A 96-well cell culture plate containing human MG-63 cells (10,000 cells per well) was cultivated for one night. Afterwards, the cells were left in contact with different concentrations of SA- 10, 25, 50, 100mM- as well as various strengths of SMF- 15, 50mT- for 24 hours. At the end of the incubation period, the conditioned medium was renewed with a fresh medium containing 0.5mg/ml MTT and incubated for four hours. Lastly, each well’s medium was disposed of, after which dmso was used to dissolve the formazan crystals and o.d reading was carried out at 570 nm. Cells that were not treated acted as a control while those that had been treated

## Real-time PCR analysis

Three days after culturing in osteogenic induction medium, the normal tissue culture. Then, they extracted total RNA using the TRizol method. Subsequently, the RNA was centrifuged and determined using the spectrophotometer at 260mm. This made it possible for the extraction of cDNA at different temperatures using the high-capacity cDNA reverse scripture. RT-PCR was done using Runx2 and Col-I specific Sybergreen, with ABI 7500 Real-Time PCR system. Fold change was calculated by means of the 2-AACq approach that provides a measure of relative mRNA expression levels.

# Statistical Analysis

These experiments were done thrice (triplicates) and presented as the mean ‡ SD, n=3. In order to measure the statistical significance, a paired t-test was carried out with the aid of the SPSS statistical program The standard of statistical significance assumed that p = 0.05 or lower.

# RESULTS

## miRNAs targeting Runx2

Figure 1 indicates the top 20 microRNAs list that are targeting runX2. Among these micro RNAs we selected microRNAs 3148 for the best set of asssesments using the mirDB database.

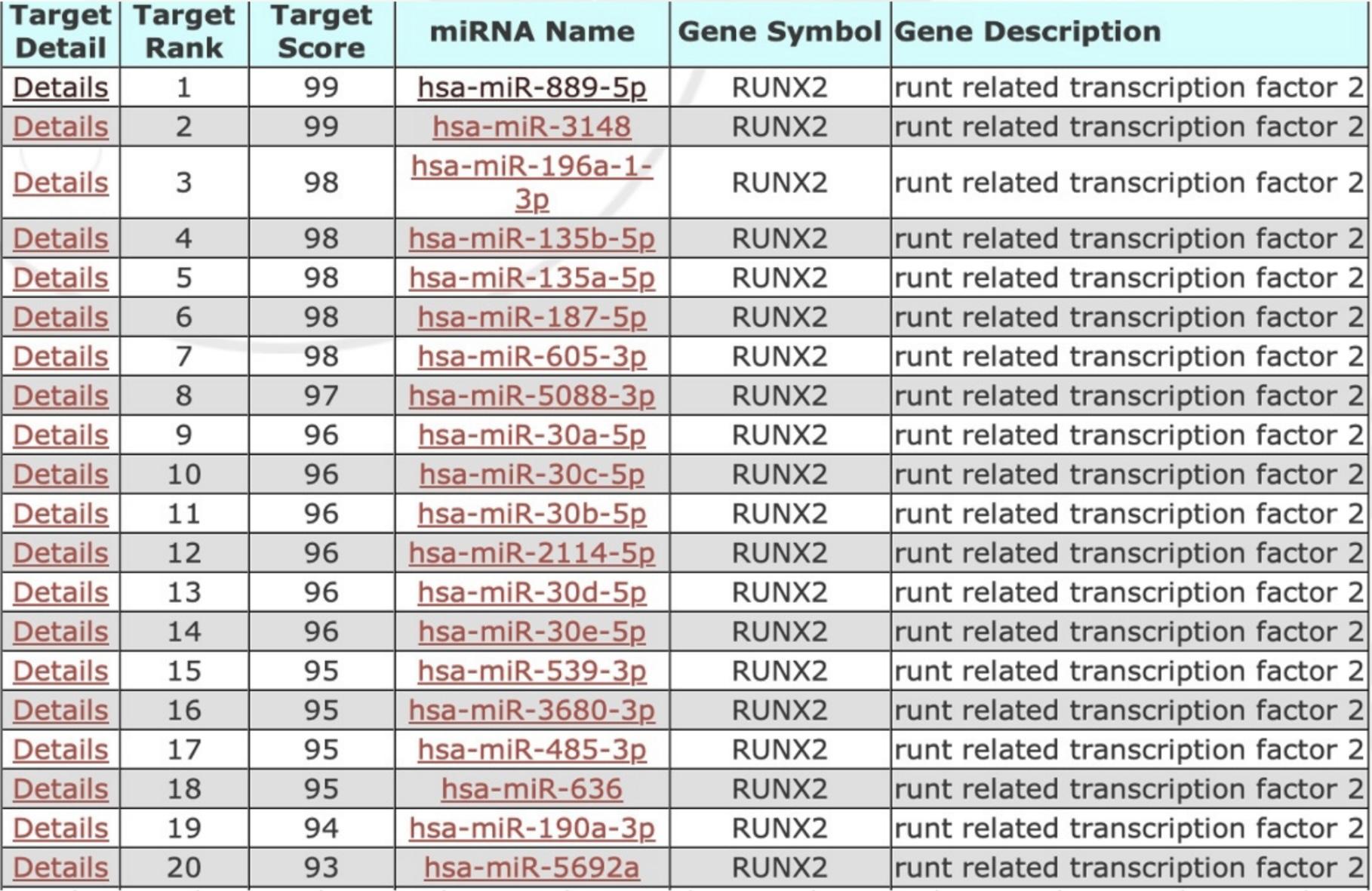


Figure 1: indicates the top 20 microRNAs list that are targeting runX2

## Predicted SEED region

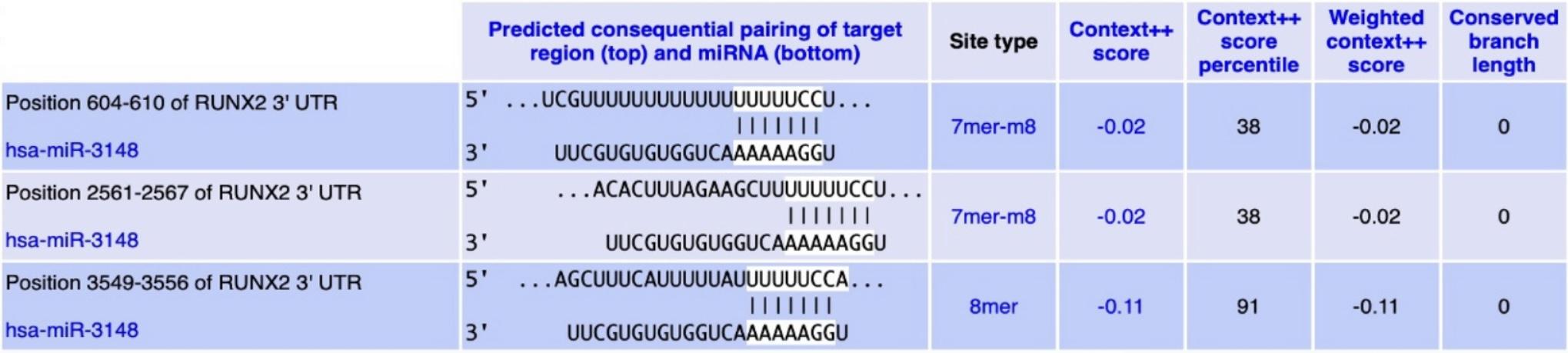


Figure 2: Predicted SEED region (paring region) present in the microRNA (miR-3148) of Runx2 UTR that were obtained from Targetscan

## runx2 expression in osteoblast after treating them with Zinc doped calcium silicate particles

We treated the cells with zinc doped calcium silicate particles. 100mg of zinc doped calcium silicate particles were immersed in 10 ml of DME for 24 hrs. After 24 hrs the medium was collected and filtered sterilized known as conditioned medium. So the cells were treated with the conditioned medium obtained from the zinc doped calcium silicate particles for a periods of 3 days under osteogenic conditions and runx2 expression was assessed

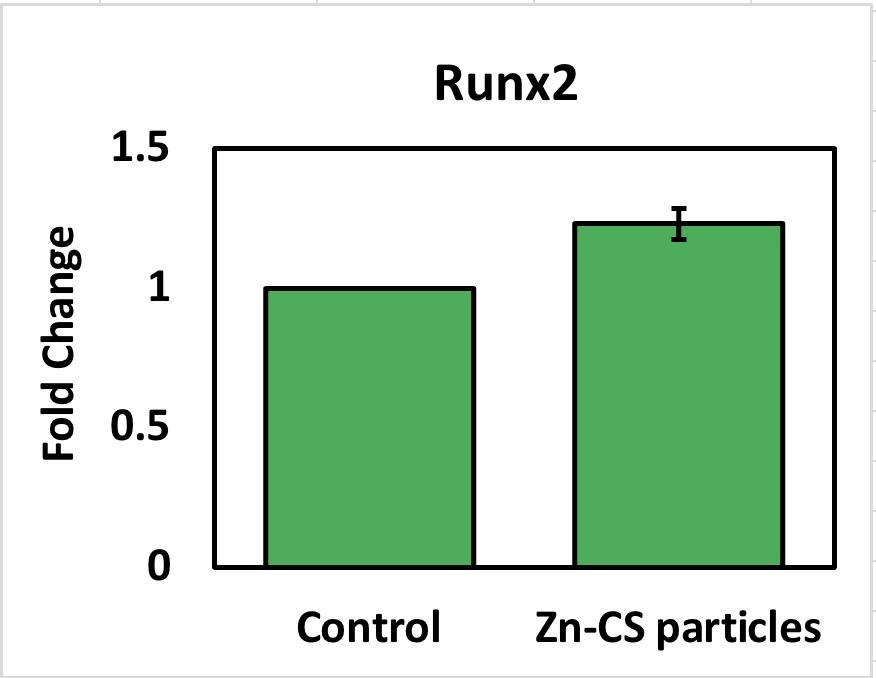


Figure 3: Effect of zinc doped calcium silicate particles on osteoblast cells.

This graph represents the runx2 expression in osteoblast after treating them with Zinc doped calcium silicate particles (Zn-Cs). Which clearly indicated that following treatment with zinc doped calcium silicate particles upregulated the runx2 expression.

## Expression of miR-3148 in osteoblasts treated with Zinc doped calcium silicate particles

We wanted to assess the expression pattern of miRNA 3148 which is supposedly targeting runx2 (negative regulator).

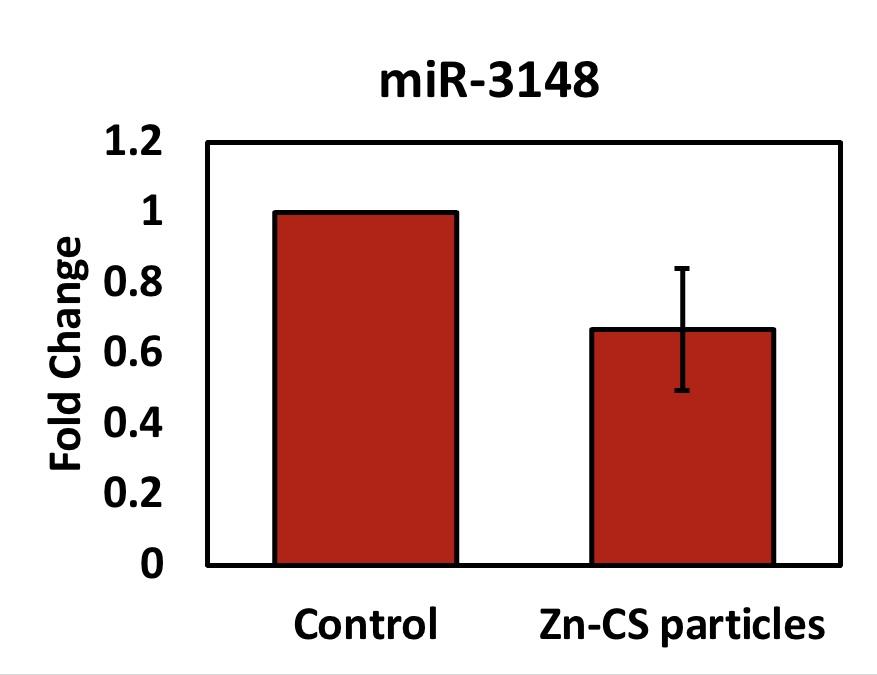


Figure 4 indicated that there was a significant decrease in the micRNA 3148 expression following the treatment with zinc doped calcium silicate particles . This clearly indicated that the increase seen in the fig 3 could be due to decrease of expression of miRNA 3148.

# DISCUSSION and CONCLUSION

Osteoblast differentiation is governed by many transcription factors, such as Runt-related transcription factor 2 (RUNX2), AMP-dependent transcription factor-4 (ATF4), and osterix (OSX), which RUNX2 thought to play a crucial role in osteoblast differentiation[(Komori, 2009)](https://paperpile.com/c/DBJKSf/taLb). Runx2 is a member of the Runx family, which includes Runx1, Runx2, and Runx3 and has the DNA-binding domain runt. Runx2 forms a heterodimer with Cbfb, resulting in increased DNA binding capacity and protein stability. P1 and P2 are the promoters for Runx2, and the transcript from P1 encodes type II Runx2 whereas the transcript from P2 encodes type I Runx2. Runx2 is found in both osteoblasts and chondrocytes. Runx2 is only modestly expressed in resting chondrocytes. Runx2 is expressed in uncommitted mesenchymal cells, and its expression is increased in preosteoblasts, peaks in immature osteoblasts, and decreases in mature osteoblasts [(Catheline et al., 2019)](https://paperpile.com/c/DBJKSf/kqql).In addition, Runx2 controls growth of osteoblast progenitors as well as their transformation into osteoblasts and bone matrix proteins synthesis (Chehelgerdi et al., 2023). A study found that Runx2 overexpression dramatically increases osteoblastic development and mineralization of bone marrow stromal cells (BMSCs) in vitro and in vivo. Similar results were found in Rat BMSCs. Further evidence suggests that Runx2-modified BMSCs may contribute to the repair of critical sized bone defects (CSBD) [(Komori, 2022; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/DBJKSf/TCVT+1IcB). Runx2 is targeted by an array of miRNAs that directly terminate its expression. miRNA is a diverse group of non-coding short RNAs which affect numerous cellular functions and signaling paths through inhibition of mRNAs. This regulation is done at the post-transcriptional level through their ability to bind with the 3′ UTR of target mRNAs, thereby influencing its stability and preventing protein synthesis. MiRNAs have also been shown through several experiments that they are important in regulating the functions of osteoblast.[(R. Hu et al., 2010)](https://paperpile.com/c/DBJKSf/QD3e)

Osteoporosis is a common disease characterized by the reduction of bone mass and deterioration of bone tissue structure, making patients more vulnerable to fractures. The development of osteoporosis has been linked to the abnormal expression of several genes, with RUNX2 being a critical transcription factor that plays a key role in regulating osteoblast differentiation and bone formation during bone development [(Khalid et al., 2008)](https://paperpile.com/c/DBJKSf/09Oo). In a study done on osteoporosis patient MiRNA-365a-3p was highly expressed and Down-regulation of miRNA-365a-3p significantly decreased the expression levels of OCN, OPN and collagen I. Furthermore, overexpression of miRNA-365a-3p markedly weakened the capability of mineralization of hBMSCs, whereas was further reversed by transfection of si-RUNX2 [(Cheng et al., 2019; Laghari et al., 2023)](https://paperpile.com/c/DBJKSf/VxYp+VMRR).According to other findings, Smurf1 (Smad ubiquitin regulatory factor 1) is a molecule that participates in ubiquitin-mediated Runx2 protein degradation and functions as a signal transduction element. It has been demonstrated that osteoblast differentiation triggered by BMP is reduced when smurf1 expression is increased in osteoblast precursor cells. Based on these findings, Bae et al. demonstrated that BMP stimulates osteoblast development by inhibiting smurf1 degradation via Runx2. In fact, the authors were able to boost BMP-induced bone formation activity by boosting Runx2 acetylation (Saadh et al., 2024). As a result, the protein becomes more stable. This strategy ubiquitination prevented Runx2 degradation [(Shen et al., 2006)](https://paperpile.com/c/DBJKSf/nxb0). Whereas in our study downregulation of Runx2 or degradation of it was prevented by treating them (osteoblast) with zinc doped calcium silicate particles.

The Zn ions can strengthen the signaling pathway and increase the expression of osteogenesis-related genes. However, high Zn ion concentrations (15 mg/L) cause Zn transporter overexpression. In addition, Si ions are required for bone formation. It was discovered that Si ions drastically promoted stem cell proliferation and osteogenic differentiation via the signaling pathways. However, high Si ion concentrations (140 mg/L) may inhibit growth. However the cells were treated with zinc doped calcium silicate particles at 0.5gm/ml in our study [(Cho & Kwun, 2018)](https://paperpile.com/c/DBJKSf/oqoi). Even though RUNX2 is thought to influence bone metabolic via different pathways, the exact way it works is not yet very well known. In conclusion , we found that osteoblasts were treated with zinc-doped calcium silicate particles under osteogenic conditions, which involved culturing the cells in a medium supplemented with vitamin C, beta-glycerol phosphate, and dexamethasone. [(*Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*, n.d.)](https://paperpile.com/c/DBJKSf/oPqf) The results showed a significant enhancement in the expression of RUNx2. This upregulation of RUNx2 was attributed to the downregulation of a specific microRNA known to target RUNx2. Here the miR-3148 was downregulated when treated with zinc-doped calcium silicate particles.

# FUTURE SCOPE

Zinc-doped calcium silicate particles are generally used for bone tissue regeneration purposes. Now we have found one such molecular mechanism behind this osteogenic formation. So from this, we can interpret similar mechanisms that could regulate Run X2 in our futuristic studies.

# REFERENCES

1. [Adel, S. M., El-Harouni, N., & Vaid, N. R. (2023). White Spot lesions: State of the art biomaterials and workflows used in prevention, progression and treatment. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/DBJKSf/NCBV)[10.1053/j.sodo.2023.01.002](http://dx.doi.org/10.1053/j.sodo.2023.01.002)
2. [*Anti-inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*. (n.d.).](http://paperpile.com/b/DBJKSf/oPqf)
3. [Arvind, T. R. P., Jain, R. K., Nagi, R., & Tiwari, A. (2022). Evaluation of alveolar bone microstructure around impacted maxillary canines using fractal analysis in Dravidian population: A retrospective CBCT study. *The Journal of Contemporary Dental Practice*, *23*(6), 593–600.](http://paperpile.com/b/DBJKSf/Y4N6) <https://www.ncbi.nlm.nih.gov/pubmed/36259297>
4. [Biomaterials mediated microRNA delivery for bone tissue engineering. (2015). *International Journal of Biological Macromolecules*, *74*, 404–412. https://doi.org/](http://paperpile.com/b/DBJKSf/wdv1)[10.1016/j.ijbiomac.2014.12.034](http://dx.doi.org/10.1016/j.ijbiomac.2014.12.034)
5. [Catheline, S. E., Hoak, D., Chang, M., Ketz, J. P., Hilton, M. J., Zuscik, M. J., & Jonason, J. H. (2019). Chondrocyte-Specific RUNX2 Overexpression Accelerates Post-traumatic Osteoarthritis Progression in Adult Mice. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, *34*(9), 1676–1689. https://doi.org/](http://paperpile.com/b/DBJKSf/kqql)[10.1002/jbmr.3737](http://dx.doi.org/10.1002/jbmr.3737)
6. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
7. [Cheng, F., Yang, M.-M., & Yang, R.-H. (2019). MiRNA-365a-3p promotes the progression of osteoporosis by inhibiting osteogenic differentiation via targeting RUNX2. *European Review for Medical and Pharmacological Sciences*, *23*(18), 7766–7774. https://doi.org/](http://paperpile.com/b/DBJKSf/VxYp)[10.26355/eurrev\_201909\_18986](http://dx.doi.org/10.26355/eurrev_201909_18986)
8. [Chokkattu, J. J., Mary, D. J., Shanmugam, R., & Neeharika, S. (2022). Embryonic toxicology evaluation of ginger- and clove-mediated titanium oxide nanoparticles-based dental varnish with zebrafish. *The Journal of Contemporary Dental Practice*, *23*(11), 1157–1162. https://doi.org/](http://paperpile.com/b/DBJKSf/Atsd)[10.5005/jp-journals-10024-3436](http://dx.doi.org/10.5005/jp-journals-10024-3436)
9. [Chokkattu, J. J., Neeharika, S., & Rameshkrishnan, M. (2023). Applications of nanomaterials in dentistry: A review. *Journal of International Society of Preventive & Community Dentistry*, *13*(1), 32–41. https://doi.org/](http://paperpile.com/b/DBJKSf/AZn8)[10.4103/jispcd.JISPCD\_175\_22](http://dx.doi.org/10.4103/jispcd.JISPCD_175_22)
10. [Cho, Y.-E., & Kwun, I.-S. (2018). Zinc upregulates bone-specific transcription factor Runx2 expression via BMP-2 signaling and Smad-1 phosphorylation in osteoblasts. *The Journal of Nutrition, Health & Aging*, *51*(1), 23–30. https://doi.org/](http://paperpile.com/b/DBJKSf/oqoi)[10.4163/jnh.2018.51.1.23](http://dx.doi.org/10.4163/jnh.2018.51.1.23)
11. [Ganapathy, D (2021). Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *International Journal of Dentistry and Oral Science*, 2976–2980. https://doi.org/](http://paperpile.com/b/DBJKSf/xB9P)[10.19070/2377-8075-21000605](http://dx.doi.org/10.19070/2377-8075-21000605)
12. [Hu, D., Li, K., Xie, Y., Pan, H., Zhao, J., Huang, L., & Zheng, X. (2016). Different response of osteoblastic cells to Mg2+, Zn2+ and Sr2+ doped calcium silicate coatings. *Journal of Materials Science. Materials in Medicine*, *27*(3), 1–13. https://doi.org/](http://paperpile.com/b/DBJKSf/cVo7)[10.1007/s10856-016-5672-y](http://dx.doi.org/10.1007/s10856-016-5672-y)
13. [Hu, R., Li, H., Liu, W., Yang, L., Tan, Y.-F., & Luo, X.-H. (2010). Targeting miRNAs in osteoblast differentiation and bone formation. *Expert Opinion on Therapeutic Targets*. https://doi.org/](http://paperpile.com/b/DBJKSf/QD3e)[10.1517/14728222.2010.512916](http://dx.doi.org/10.1517/14728222.2010.512916)
14. [Jadlowiec, J. A., Celil, A. B., & Hollinger, J. O. (2003). Bone tissue engineering:recent advances and promising therapeutic agents. *Expert Opinion on Biological Therapy*. https://doi.org/](http://paperpile.com/b/DBJKSf/jFOS)[10.1517/14712598.3.3.409](http://dx.doi.org/10.1517/14712598.3.3.409)
15. [Jain, R. K., & Verma, P. (2022). Visual assessment of extent of White Spot lesions in subjects treated with fixed orthodontic appliances: A retrospective study. *World Journal of Dentistry*, *13*(3), 245–249. https://doi.org/](http://paperpile.com/b/DBJKSf/onfi)[10.5005/jp-journals-10015-2042](http://dx.doi.org/10.5005/jp-journals-10015-2042)
16. [Khalid, O., Baniwal, S. K., Purcell, D. J., Leclerc, N., Gabet, Y., Stallcup, M. R., Coetzee, G. A., & Frenkel, B. (2008). Modulation of Runx2 Activity by Estrogen Receptor-α: Implications for Osteoporosis and Breast Cancer. *Endocrinology*, *149*(12), 5984–5995. https://doi.org/](http://paperpile.com/b/DBJKSf/09Oo)[10.1210/en.2008-0680](http://dx.doi.org/10.1210/en.2008-0680)
17. [Komori, T. (2009). Regulation of Osteoblast Differentiation by Runx2. *Osteoimmunology*, 43–49. https://doi.org/](http://paperpile.com/b/DBJKSf/taLb)[10.1007/978-1-4419-1050-9\_5](http://dx.doi.org/10.1007/978-1-4419-1050-9_5)
18. [Komori, T. (2022). Whole Aspect of Runx2 Functions in Skeletal Development. *International Journal of Molecular Sciences*, *23*(10), 5776. https://doi.org/](http://paperpile.com/b/DBJKSf/TCVT)[10.3390/ijms23105776](http://dx.doi.org/10.3390/ijms23105776)
19. [Laghari, I. A., Pandey, A. K., Samykano, M., Aljafari, B., Kadirgama, K., Sharma, K., & Tyagi, V. V. (2023). Thermal energy harvesting of highly conductive graphene-enhanced paraffin phase change material. *Journal of Thermal Analysis and Calorimetry*, *148*(18), 9391–9402. https://doi.org/](http://paperpile.com/b/DBJKSf/VMRR)[10.1007/s10973-023-12336-5](http://dx.doi.org/10.1007/s10973-023-12336-5)
20. [Marya, A., Venugopal, A., Karobari, M. I., & Rokaya, D. (2022). White Spot lesions: A serious but often ignored complication of orthodontic treatment. *The Open Dentistry Journal*, *16*(1). https://doi.org/](http://paperpile.com/b/DBJKSf/OIih)[10.2174/18742106-v16-e2202230](http://dx.doi.org/10.2174/18742106-v16-e2202230)
21. [Meenakshi, S. S., & Sankari, M. (2021). Effectiveness of chitosan nanohydrogel as a bone regenerative material in intrabony defects in patients with chronic periodontitis: A randomized clinical trial. *Journal of Advanced Oral Research*, *12*(2), 222–228. https://doi.org/](http://paperpile.com/b/DBJKSf/B96y)[10.1177/2320206821998574](http://dx.doi.org/10.1177/2320206821998574)
22. [Merchant, A., Ganapathy, D. M., & Maiti, S. (2022). Effectiveness of local and topical anesthesia during gingival retraction. *Brazilian Dental Science*, *25*(1), e2591. https://doi.org/](http://paperpile.com/b/DBJKSf/ttka)[10.4322/bds.2022.e2591](http://dx.doi.org/10.4322/bds.2022.e2591)
23. [MicroRNA function in craniofacial bone formation, regeneration and repair. (2021). *Bone*, *144*, 115789. https://doi.org/](http://paperpile.com/b/DBJKSf/w0o4)[10.1016/j.bone.2020.115789](http://dx.doi.org/10.1016/j.bone.2020.115789)
24. [Muthuswamy Pandian, S., Subramanian, A. K., Ravikumar, P. A., & Adel, S. M. (2022). Biomaterial testing in contemporary orthodontics: Scope, protocol and testing apparatus. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/DBJKSf/1IcB)[10.1053/j.sodo.2022.12.011](http://dx.doi.org/10.1053/j.sodo.2022.12.011)
25. [Padmanabhan, V. P., Sivashanmugam, P., Kulandaivelu, R., Sagadevan, S., Sridevi, B., Govindasamy, R., & Thiruvengadam, M. (2022). Biosynthesised silver nanoparticles loading onto biphasic calcium phosphate for antibacterial and bone tissue engineering applications. *Antibiotics (Basel, Switzerland)*, *11*(12), 1780. https://doi.org/](http://paperpile.com/b/DBJKSf/gCyK)[10.3390/antibiotics11121780](http://dx.doi.org/10.3390/antibiotics11121780)
26. [Pandiyan, I., Sri, S. D., Indiran, M. A., Rathinavelu, P. K., Prabakar, J., & Rajeshkumar, S. (2022). Antioxidant, anti-inflammatory activity of Thymus vulgaris-mediated selenium nanoparticles: An in vitro study. *Journal of Conservative Dentistry: JCD*, *25*(3), 241–245. https://doi.org/](http://paperpile.com/b/DBJKSf/oO4m)[10.4103/JCD.JCD\_369\_21](http://dx.doi.org/10.4103/JCD.JCD_369_21)
27. [Ramamurthy, S., Thiagarajan, K., Varghese, S., Kumar, R., Karthick, B. P., Varadarajan, S., & Balaji, T. M. (2022). Assessing the in vitro antioxidant and anti-inflammatory activity of Moringa oleifera crude extract. *The Journal of Contemporary Dental Practice*, *23*(4), 437–442. https://doi.org/](http://paperpile.com/b/DBJKSf/CDU6)[10.5005/jp-journals-10024-3323](http://dx.doi.org/10.5005/jp-journals-10024-3323)
28. Saadh, M. J., Rasulova, I., Almoyad, M. A. A., Kiasari, B. A., Ali, R. T., Rasheed, T. & Ciongradi, C. I. (2024). Recent progress and the emerging role of lncRNAs in cancer drug resistance; focusing on signaling pathways. Pathology-Research and Practice, 253, 154999.
29. [Shen, R., Chen, M., Wang, Y.-J., Kaneki, H., Xing, L., O’Keefe, R. J., & Chen, D. (2006). Smad6 Interacts with Runx2 and Mediates Smad Ubiquitin Regulatory Factor 1-induced Runx2 Degradation \*. *The Journal of Biological Chemistry*, *281*(6), 3569–3576. https://doi.org/](http://paperpile.com/b/DBJKSf/nxb0)[10.1074/jbc.M506761200](http://dx.doi.org/10.1074/jbc.M506761200)
30. [Solanki, L. A., Dinesh, S. P. S., Jain, R. K., & Balasubramaniam, A. (2023). Effects of titanium oxide coating on the antimicrobial properties, surface characteristics, and cytotoxicity of orthodontic brackets - A systematic review and meta analysis of in-vitro studies. *Journal of Oral Biology and Craniofacial Research*, *13*(5), 553–562. https://doi.org/](http://paperpile.com/b/DBJKSf/Srk9)[10.1016/j.jobcr.2023.05.014](http://dx.doi.org/10.1016/j.jobcr.2023.05.014)
31. [Sreevarun, M., Ajay, R., Suganya, G., Rakshagan, V., Bhanuchander, V., & Suma, K. (2023). Formulation, configuration, and physical properties of dental composite resin containing a novel 2π + 2π photodimerized crosslinker - cinnamyl methacrylate: An in vitro research. *The Journal of Contemporary Dental Practice*, *24*(6), 364–371. https://doi.org/](http://paperpile.com/b/DBJKSf/4RKx)[10.5005/jp-journals-10024-3480](http://dx.doi.org/10.5005/jp-journals-10024-3480)
32. [Subramaniam, R., Vijakumaran, U., Shanmuganantha, L., Law, J.-X., Alias, E., & Ng, M.-H. (2023). The Role and Mechanism of MicroRNA 21 in Osteogenesis: An Update. *International Journal of Molecular Sciences*, *24*(14), 11330. https://doi.org/](http://paperpile.com/b/DBJKSf/AKRo)[10.3390/ijms241411330](http://dx.doi.org/10.3390/ijms241411330)
33. [Subramanian, A., & Harikrishnan, S. (2023). 3D printing in orthodontics: A narrative review. *Journal of International Oral Health: JIOH*, *15*(1), 15. https://doi.org/](http://paperpile.com/b/DBJKSf/8Yvx)[10.4103/jioh.jioh\_83\_22](http://dx.doi.org/10.4103/jioh.jioh_83_22)
34. [Sugumaran, S., Selvam, D., Nivedhitha, M. S., Ganesh Mohanraj, K., Almutairi, B. O., Arokiyaraj, S., Guru, A., & Arockiaraj, J. (2023). Role of individual and combined impact of simvastatin and α-TCP in rat calvarial bone defect: An experimental study. *The Saudi Dental Journal*, *35*(7), 861–868. https://doi.org/](http://paperpile.com/b/DBJKSf/1quE)[10.1016/j.sdentj.2023.07.013](http://dx.doi.org/10.1016/j.sdentj.2023.07.013)
35. [Wadhwani, V., Sivaswamy, V., & Rajaraman, V. (2022). Surface roughness and marginal adaptation of stereolithography versus digital light processing three-dimensional printed resins: An in-vitro study. *Journal of Indian Prosthodontic Society*, *22*(4), 377–381. https://doi.org/](http://paperpile.com/b/DBJKSf/zpme)[10.4103/jips.jips\_8\_22](http://dx.doi.org/10.4103/jips.jips_8_22)
36. [Wang, J., Cui, Y., Liu, H., Li, S., Sun, S., Xu, H., Peng, C., Wang, Y., & Wu, D. (2022). MicroRNA-loaded biomaterials for osteogenesis. *Frontiers in Bioengineering and Biotechnology*, *10*, 952670. https://doi.org/](http://paperpile.com/b/DBJKSf/lFuW)[10.3389/fbioe.2022.952670](http://dx.doi.org/10.3389/fbioe.2022.952670)
37. [Yuan, X., Wu, T., Lu, T., & Ye, J. (2023). Effects of Zinc and Strontium Doping on In Vitro Osteogenesis and Angiogenesis of Calcium Silicate/Calcium Phosphate Cement. *ACS Biomaterials Science & Engineering*. https://doi.org/](http://paperpile.com/b/DBJKSf/eG92)[10.1021/acsbiomaterials.3c00193](http://dx.doi.org/10.1021/acsbiomaterials.3c00193)