Evaluating the Osteogenic Properties of Cu-Zn Alloy Nanoparticles Intended for Bone Formation

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**Abstract:** Orthopaedics faces difficult problems as a result of congenital anomalies, disease, or bone defects caused by trauma. Tissue engineering techniques use biomaterials to encourage bone regeneration and repair, and they present intriguing possibilities. Due to its beneficial qualities, including good biocompatibility, antibacterial activity, and tunable mechanical properties, Cu-Zn alloy nanoparticles have attracted attention. However, to assess their suitability for bone tissue engineering applications, a complete understanding of their osteogenic potential is essential. To evaluate the osteogenic properties of cu zn alloy nanoparticles intended for bone formation. Nanoparticles were first taken in PBS(phosphate buffered saline) and they were treated to human osteoblast cells MG-63 cells where produced from NCCS(National Centre for Cell Science) in Pune. The cells were exposed to this nanoparticle of a particular concentration for a period of 10 days under an osteogenic medium. At the end of 10 days we collected mRNA converted to cDNA and we assessed the expression pattern of Runx2, collagen, and ALP to real-time PCR. After treatment (Real-time PCR) we got the experimental pattern of all the osteoblastic markers gene Runx2, ALP, and Collagen. Significant increase in Runx2 expression level when compared to untreated cells. The Cu-Zn alloy nanoparticles to promote Runx2 expression. A similar pattern was also observed for ALP but there was no change in the collagen 1 expression. It indicates that ALP, as well as Runx2 mRNA expression, were related in the presence of Cu-Zn alloy nanoparticles when compared to the control. This study highlights the potential of Cu-Zn alloy nanoparticles as a bone tissue engineering material by shedding light on their osteogenic capabilities. The research confirms their practical applicability in stimulating bone regeneration and repair, which will progress orthopedics.

**Keywords**: Cu-Zn alloy nanoparticles, Bone Formation, Osteoblast proliferation, Osteoblast differentiation, Biocompatibility, Cytotoxicity, Bone tissue engineering, Nanoparticles characterization,

# INTRODUCTION

Innovation and multidisciplinary research have excitingly come together in the field of biomaterials for bone regeneration, searching for new approaches to tackle the intricate problems of bone tissue engineering.[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/xorWbb/ygwn+q9d4+rQle) Copper-zinc (Cu-Zn) alloy nanoparticles have become a unique and promising contender among the many materials being studied; they provide a synergistic interaction of vital trace elements with established roles in bone metabolism.[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/xorWbb/sKBM+93hL) Bone's extraordinary ability to regenerate itself depends on carefully regulated interactions among cells, signaling molecules, and the extracellular matrix (ECM). [(“Distribution and Evaluation of Bone and Soft Tissue Tumors Operated in a Tertiary Care Center,” 2019)](https://paperpile.com/c/xorWbb/ROWy2)Collagen type I is a byproduct of the activity of osteoblasts, which are the principal builders of bone.[(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/xorWbb/zb8u+TfoF) Osteoblasts also coordinate the synthesis of other ECM components..[(Marya et al., 2022)](https://paperpile.com/c/xorWbb/Ycgq) Within this framework, the incorporation of Cu-Zn alloy nanoparticles into the complex dance of bone formation has the capacity to regulate osteoblastic activities and, as a result, impact the course of regeneration. [(“Fabrication, in Vitro and in Vivo Properties of Porous Zn–Cu Alloy Scaffolds for Bone Tissue Engineering,” 2022)](https://paperpile.com/c/xorWbb/p51em)

A vital trace element in the human body, copper is involved in many physiological processes, such as the synthesis of collagen, the formation of bones, and antioxidant defense systems. Another crucial trace element, zinc, is involved in many biological processes like wound healing, immunological response, and enzymatic activity. [(Zhao et al., 2023)](https://paperpile.com/c/xorWbb/NVRFy)It is also essential for the growth and upkeep of bones. One essential stage in the creation of the bone matrix is collagen synthesis, which is aided by copper. Cu-Zn alloy nanoparticles may improve osteoblast function and bone formation by releasing copper under controlled conditions.[(Wang, 2012)](https://paperpile.com/c/xorWbb/GyDOX)

Cu-Zn alloy nanoparticles are being studied because of the elemental composition's inherent relationship to vital biological functions.[(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/xorWbb/Ycgq+9Nnu) An alloy that may enhance the osteogenic potential in the regenerative niche is composed of zinc, which is essential for matrix metalloproteinase activity, and copper, which is a necessary cofactor for enzymes involved in collagen cross-linking. [(Zhuang et al., 2020)](https://paperpile.com/c/xorWbb/GyUWX)Copper has gained attention for its role in promoting an environment that is favorable to bone regeneration. Copper is known for its angiogenic and antimicrobial properties.[(Sreevarun et al., 2023)](https://paperpile.com/c/xorWbb/jsSp)

In addition to its direct role in collagen synthesis, copper has a variety of other effects, including angiogenesis, which is an essential process for the formation of a vascular network in growing bone tissues.[(Wadhwani et al., 2022)](https://paperpile.com/c/xorWbb/mhgZ) Zinc adds another level of complexity and potential to the alloy concurrently. [(“Copper-Containing Mesoporous Bioactive Glass Nanoparticles as Multifunctional Agent for Bone Regeneration,” 2017)](https://paperpile.com/c/xorWbb/QtSoU)Zinc is essential for maintaining the delicate balance between bone formation and resorption because of its roles in immune system function, enzymatic activity, and bone mineralization.[(Solanki et al., 2023)](https://paperpile.com/c/xorWbb/Pwt4)

When copper-zinc (Cu-Zn) alloy nanoparticles are compared to other elements or materials, certain features stand out, making them desirable for specific applications. [(Bozorgi et al., 2021)](https://paperpile.com/c/xorWbb/nILay)These nanoparticles' combination of zinc and copper has a synergistic effect. Zinc promotes enzymatic activity and bone mineralization, while copper offers antimicrobial and angiogenic qualities. Cu-Zn alloys are distinguished from materials with singular properties by this dual functionality. [(Bozorgi et al., 2021)](https://paperpile.com/c/xorWbb/nILay)

The investigation's specific focus is on how extracellular matrix formation, mineralization, angiogenesis, and osteoblast proliferation and differentiation are impacted by Cu-Zn alloy nanoparticles.[(Ganapathy 2021)](https://paperpile.com/c/xorWbb/4nDh) To assure their safety and effectiveness for bone tissue engineering applications, the study also intends to evaluate the biocompatibility and potential cytotoxicity of Cu-Zn alloy nanoparticles.[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/xorWbb/jxVl) The study hopes to advance knowledge of the potential of Cu-Zn alloy nanoparticles as a bone tissue engineering material and their potential therapeutic relevance for fostering bone regeneration and repair by attaining these goals.

# MATERIALS AND METHODS

Nanoparticles were first taken in PBS(phosphate buffered saline) and they were treated to human osteoblast cells MG-63 cells where produced from NCCS(National Centre for Cell Science) in Pune. The cells were exposed to this nanoparticle of a particular concentration for 10 days under an osteogenic medium. At the end of 10 days we collected mRNA converted to cDNA and we assessed the expression pattern of Runx2, collagen, and ALP to real-time PCR.

# CELL CULTURE

Human osteoblastic cells (MG-63) were kept in regular culture conditions after being purchased from NCCS (National Center for Cell Sciences), Pune. After being initially passaged, the cells were used in additional cell culture studies. To conduct gene expression studies for osteogenic differentiation marker genes, cells were cultured under standard conditions and supplemented with osteogenic induction medium, which contains beta-glycerophosphate, dexamethasone, and vitamin C.

# REAL-TIME PCR ANALYSIS

For ten days, human osteoblastic cells were cultured normally in an osteogenic induction medium. The TRIzol method was then used to extract total RNA. A spectrophotometer was used to measure the concentration of the extracted RNA at 260 m after it had been purified by centrifugation. Next, the High-Capacity cDNA reverse transcription kit was used at particular temperature steps to convert the purified RNA to complementary DNA (CDNA). Using SYBR green and the appropriate primers for the target genes (Runx2 and Col-I), RT-PCR was carried out on the ABI 7500 Real-time PCR system. The 2-AACq method was utilized to ascertain the relative mRNA expression levels, yielding fold change values as the result.

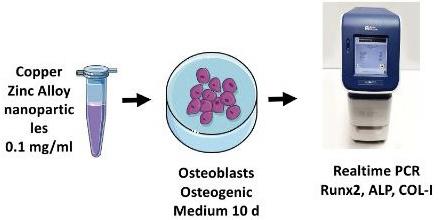


Figure 1: Incubation

Initially cu zn alloy nanoparticles 0.1 g were immersed in 10 ml of 10 percent FBS-containing medium this is called condition medium. conditioned medium was treated to cells for a time point of 10 days. Following this incubation real-time PCR was carried out for runx2, alkaline phosphatase and ,collagen 1 genes.

## STATISTICAL ANALYSIS

The experiments were repeated three times (triplicate), and the results were presented as the mean standard deviation (SD), with a sample size (n) of 3. To assess the statistical significance, a paired t-test was conducted using the SPSS statistical software. A p-value equal to or less than 0.05 was considered to indicate statistical significance.

# RESULTS

In order to assess the osteogenic properties of cu zn alloy nanoparticles,initially we treated the human osteoblast for the period of 10 days under osteogenic medium in the presence or absence of cu zn alloy nanoparticles condition medium.

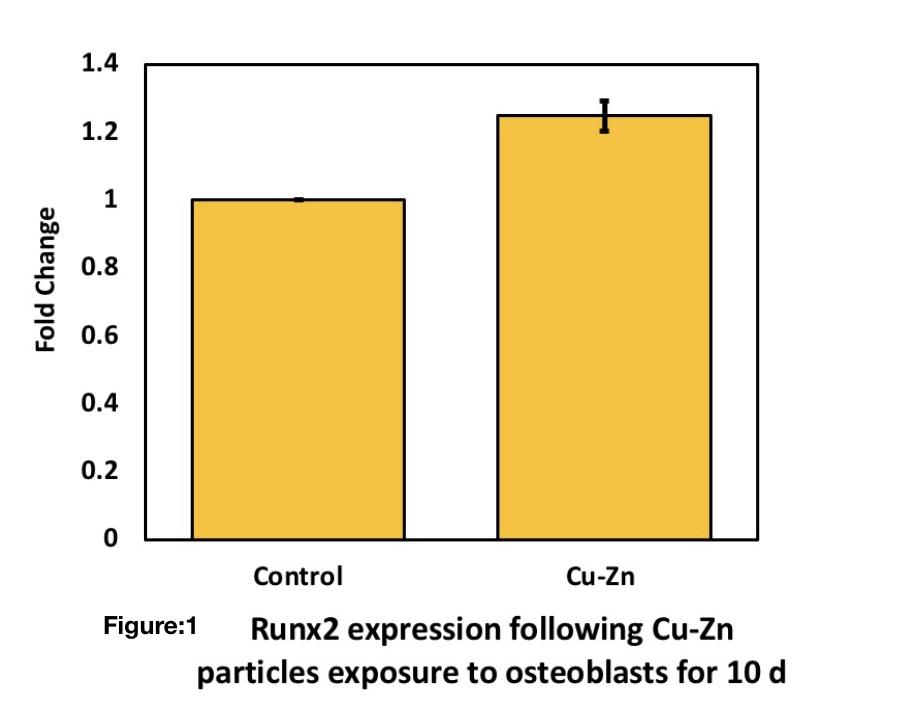


Figure 2: Particles exposure

The result clearly indicated that there was significant increase in Runx2 expression following the Cu-Zn alloy nanoparticles exposure.

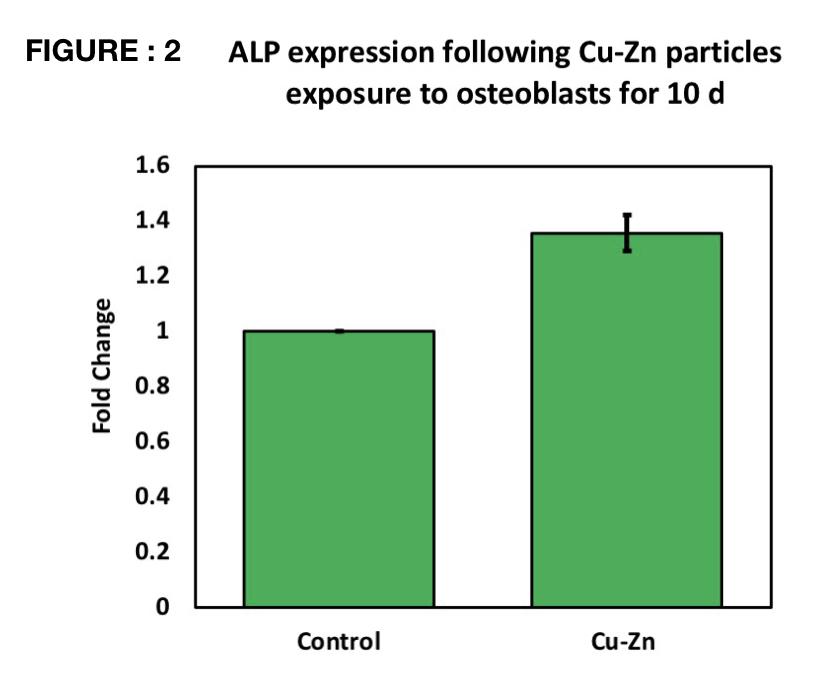


Figure 3: Expression

We assessed the alkaline phosphatase expression for a similarly significant increase in Runx2 expression level when compared to untreated cells.

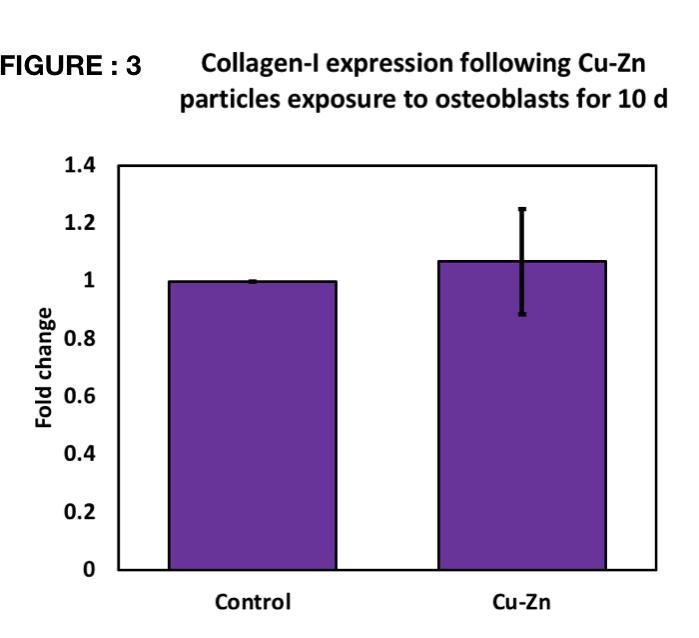


Figure 4 indicates the collagen 1 expression among control and treated cells. The result indicated that there was no significant change in the collagen 1 expression; both control and treated cells exhibited a similar kind of collagen 1 expression.

# DISCUSSION

After treatment (Real-time PCR) we got the experimental pattern of all the osteoblastic markers gene Runx2, ALP, and Collagen. Significant increase in Runx2 expression level when compared to untreated cells.[(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/xorWbb/e3Lw+cATU). The Cu-Zn alloy nanoparticles promote Runx2 expression. A similar pattern was also observed for ALP but there was no change in the collagen 1 expression. It indicates that ALP, as well as Runx2 mRNA expression, were related in the presence of Cu-Zn alloy nanoparticles when compared to the control.

Runx2, a transcription factor also referred to as Core-binding factor subunit alpha-1 (Cbfa1), is essential for controlling gene expression during osteoblast differentiation and bone formation. It belongs to the family of transcription factors that contain runt domains. As a transcriptional regulator, Runx2 regulates the expression of genes that are involved in a variety of biological processes.[(Lian & Stein, 2003)](https://paperpile.com/c/xorWbb/d7FHj)One of the first steps in osteogenesis is the commitment of mesenchymal stem cells to the osteoblastic lineage, which is dependent on Runx2.Runx2 is essential for healthy skeletal growth.[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/xorWbb/fMwD) Mice with non-functional Runx2 show no bone formation at all. Numerous genes essential for osteoblast function, such as those involved in extracellular matrix formation and mineralization, are regulated by Runx2.[(Wei et al., 2015)](https://paperpile.com/c/xorWbb/LGxkf)

The alkaline phosphatase enzyme family is responsible for catalyzing the release of inorganic phosphate from a variety of substrates by hydrolyzing phosphate esters in an alkaline environment. That's why it's called "alkaline"—pH levels above 7 are ideal for it to function. In particular, osteoblasts are linked to ALP. It is critical for bone mineralization and an important marker of early osteoblastic activity.[(“Bone-Alkaline Phosphatase as Indicator of Bone Formation,” 1991)](https://paperpile.com/c/xorWbb/CW0O9) By encouraging the hydrolysis of pyrophosphate, an inhibitor of mineralization, ALP takes part in the control of extracellular matrix mineralization.It plays a role in the metabolism of nucleotides and is necessary for healthy bile production, liver function, and normal bone mineralization. An increase in ALP expression may be observed as a result of the beneficial influence of Cu-Zn alloy nanoparticles on osteogenic differentiation. Increased osteoblast activity and elevated ALP levels indicate a favorable environment for bone formation.[(Siffert, 1951)](https://paperpile.com/c/xorWbb/SCtiL)

A crucial extracellular matrix protein known as collagen type I (Col I) is a marker of mature osteoblast function and is essential to the formation of bone with higher levels of collagen expression I propose that Cu-Zn alloy nanoparticles have a beneficial effect on the synthesis of extracellular matrix proteins. [(Garnero, 2015)](https://paperpile.com/c/xorWbb/EVNEY) Since Collagen I gives bone tissue its structural integrity, this stage of the bone-formation process is crucial. One important factor maintaining the structural integrity of bone tissue is collagen I. A rise in its expression suggests that the nanoparticles might aid in the development of a strong and well-organized bone matrix.[(Santosh Kumar et al., 2023)](https://paperpile.com/c/xorWbb/2aYZ)

The ability of Cu-Zn alloy nanoparticles to promote osteoblast proliferation and differentiation is one of the main findings from investigations. Osteoblasts produce and deposit components of the extracellular matrix, which is essential for the development of bones. It has been demonstrated that Cu-Zn alloy nanoparticles encourage osteoblast growth, increasing the amount of cells accessible for matrix creation. Additionally, it has been discovered that these nanoparticles promote osteoblastic development, as shown by an uptick in the production of osteogenic markers such as alkaline phosphatase, collagen type I, and osteocalcin. These results imply that Cu-Zn alloy nanoparticles may hasten bone remodeling and forming processes. [(Bahnasawy et al., 2022)](https://paperpile.com/c/xorWbb/qb5Zr)

The production of the extracellular matrix and its subsequent mineralization are crucial steps in the process of bone formation. Cu-Zn alloy nanoparticles have shown they can stimulate osteoblasts' production of extracellular matrices(Chehelgerdi et al., 2023). Improved matrix deposition can result from these nanoparticles' ability to upregulate the production of matrix proteins including collagen and fibronectin. [(Forero et al., 2017)](https://paperpile.com/c/xorWbb/9ESBa)Additionally, it has been demonstrated that Cu-Zn alloy nanoparticles improve mineralization, promoting the development of hydroxyapatite crystals, which are crucial for bone stiffness and strength. Cu-Zn alloy nanoparticles' stimulation of extracellular matrix production and mineralization emphasizes its osteoinductive potential and capacity to improve bone tissue regeneration.[(Gaharwar et al., 2013)](https://paperpile.com/c/xorWbb/5Sgiv)

Consistent with research on titanium and hydroxyapatite nanoparticles, our results show beneficial effects on osteoblast differentiation and proliferation. Comparison: Our findings support the idea that Cu-Zn alloys have the potential for bone tissue engineering when compared to research on well-established osteogenic materials. The angiogenic effects that have been observed align with research on nanoparticles of silver and gold,[(Kumar et al., 2019)](https://paperpile.com/c/xorWbb/4PkVO) indicating a wider pattern of metallic nanoparticles impacting angiogenesis. Contrast: Consistent with results from other metallic nanoparticles highlights the possible importance of this characteristic in facilitating vascularization during the process of bone regeneration.

One of the most important elements influencing the nanoparticles' osteogenic properties is the release of Cu and Zn ions from them. According to certain studies, copper ions can promote angiogenesis, which is essential for the growth of new blood vessels in bone tissue that is still forming. Oxygen and nutrients required for osteoblast function and bone growth are provided via angiogenesis. [(Lin et al., 2017)](https://paperpile.com/c/xorWbb/9AFXd)Cu ions also possess antibacterial qualities that guard against infection at the site of bone repair. On the other hand, Zn ions have been demonstrated to support osteoblast development and mineralization and play a significant role in bone metabolism (Saadh et al., 2024). Therefore, bone regeneration can be improved and the entire osteogenic process can be supported by the regulated release of Cu and Zn ions from Cu-Zn alloy nanoparticles.[(Boccaccini et al., 2021; Lin et al., 2017)](https://paperpile.com/c/xorWbb/9AFXd+IXso2)

# CONCLUSION

Cu-Zn alloy nanoparticles hold great promise for promoting bone formation and regeneration. Their unique properties, including biocompatibility, mechanical strength, controlled degradation, and ion release, make them attractive candidates for orthopedic applications. Continued research and development in this field will pave the way for innovative strategies to address bone disorders, fractures, and other orthopedic challenges, ultimately improving patient outcomes in the field of bone tissue engineering.

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