Influence of green synthesized (Rutin) copper oxide nanoparticles impregnated scaffold on the expression of osteocalcin and type 1 collagen

Nivedha Valliammai Mahalingam [1] , *Nidhita [2], Karthikeyan Murthykumar [3]

1) CRRI,Saveetha Dental College,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University, Chennai,151901047.sdc@saveetha.com

2)Senior Lecturer,Department of Periodontics ,Saveetha Dental College, Saveetha University,
Saveetha Institute of Medical and Technical Sciences.
nidhitas.sdc@saveetha.com
3)Senior Lecturer,Department of Periodontics,Saveetha Dental College
Saveetha institute of medical and technical sciences,karthikeyanmurthykumar@gmail.com
8939796439

Corresponding author:Dr.Nidhita

Senior Lecturer,Department of Periodontics,Saveetha Dental College, Saveetha University,
Saveetha Institute of Medical and Technical Sciences
nidhitas.sdc@saveetha.com

Abstract

Background: Bone tissue engineering aims to regenerate bone defects using biocompatible materials and bioactive agents. Nanoparticles offer significant advantages by enhancing cell-material interactions, delivering therapeutic molecules, and modulating biological responses.

Methods: In this study, scaffolds were fabricated using a blend of hyaluronic acid, carrageenan, and gelatin. Rutin-doped copper oxide nanoparticles were incorporated into the scaffold matrix. The osteogenic potential of these scaffolds was evaluated by assessing cell proliferation, differentiation, and gene expression of osteogenic markers (osteocalcin and collagen type I).

Results: The results demonstrated enhanced osteogenic gene expression in cells cultured on the copper oxide-containing scaffolds compared to controls. This suggests that copper oxide nanoparticles facilitated cell adhesion, proliferation, and promoted osteogenic differentiation.

Conclusion: The findings indicate that the incorporation of rutin-doped copper oxide nanoparticles into the scaffolds significantly improved their osteogenic properties. These results suggest the potential of these novel scaffolds for bone tissue regeneration applications.

Keywords: Bone tissue engineering, Nanoparticles, Copper oxide, Scaffolds, Osteogenesis, Regeneration

INTRODUCTION:

The initiation of nanoparticles into bone tissue engineering strategies is advantageous to preside cell fate into osteogenesis and the regeneration of large bone defects. Being multifunctional compounds, nanoparticles contribute to scaffold-free and scaffold-based tissue engineering strategies to improve osteogenesis and bone regeneration[1].They stabilize the inflammatory responses and osteo/angio/osteoclastic signaling pathways to generate an osteogenic niche. As well, nanoparticles interact with biomolecules, enhancing their half-life and bioavailability. Nanoparticles are promising technology to promote osteogenesis. Nevertheless, the interaction of nanoparticles with the biological milieu is complicated, and more considerations need to be provided on the employment of nanoparticles in clinical applications because of Nanoparticles-induced toxicities.[2]

The tissue and organ transplantation has few drawbacks such as limited donor availability, the need for immunosuppression and insufficient success rate (rejection of the transplant), has developed the space for an increasing demand in tissue engineering and regenerative medicine (TERM) solutions, a rapidly growing multidisciplinary field. The merging of the biological, material and engineering sciences to develop and manufacture artificial structures that resemble the native tissue/organ not only as implantable systems but also as model, miniaturized organs. Impersonating the natural extracellular matrix (ECM) composition of a tissue through constructing a three dimensional (3D) scaffold for cells with appropriate mechanical strength, ease of monitoring cellular activities and delivering of bioactive agents require a nanoscale approach rather than a macroscopic one to obtain satisfactory results. Nanoparticles (NPs) are considered to provide high control over properties of scaffolds such as tuning their mechanical strength and providing controlled release of bioactive agents. In Addition, drawbacks and limition factors such as low solubility, unstable bioactivity and short circulation half-life of bioactive molecules (growth factors, cytokines, inhibitors, genes, drugs etc.) and contrast agents have made the NPs as one of the most suitable candidates for bioactive agent delivery and monitoring for applications.[3]

Nanotechnology viewing as a processing technology includes synthesizing NPs and using them for a wide range of applications. NPs with sizes ranging between ~ 10 to 1,000 nm can be prepared in solid and colloidal forms. [4]NPs has a vast area of applications in the production of sensors, photovoltaic devices, and biomedical field such as drugs delivery and vaccine adjuvants. The impact of nanotechnology has altered traditional and simple approaches in TERM toward more complex and efficient systems. Together with NPs, other products of nanoscale technology such as nanofibers and nanopatterned surfaces have been used for directing cell behavior in the TERM field.[5] Utilization of simultaneous therapeutic and imaging systems, embedding novel biomaterials with superior spatiotemporal control within scaffolds, modulating release of multiple bioactive agents especially growth factors to direct fate of stem cells and morphogenesis, adjusting mechanical strength of scaffolds for hard tissue applications, and minimizing toxicity and increasing biocompatibility through tissue specific delivery are among various applications of NPs in TERM. Nanoparticles can be concoted with various types of materials such as ceramics, metals, natural and synthetic polymers. The compositions and characteristic advantages like high penetration ability, high surface area with tunable surface properties make them as one of the widely preferred candidates in TERM field for imaging, mechanical strength enhancement, as bioink supplements, antimicrobial, and bioactive agent carriers[6].Previously our department has published extensive research on various aspects which inspired us to do this study.[7–10][11]

Obtaining the planting material of the woody plants by clonal micropropagation is the modern intensive method of mass asexual reproduction in tissue and cell culture, where the resulting plants are genetically identical to the original specimen. Subsequently the culture media provide the ideal conditions for the growth of microorganisms, plant tissue cultures are to be produced and maintained under aseptic conditions.[12] Antibiotics are used widely to reduce the risk of contamination in plants propagated in vitro. However, beside the bactericidal effect, antibiotics can have a toxic effect on plant tissues and inhibit the growth and development of explants. Additionally, it is known that microorganisms can adapt to biocidal drugs by mutations, which leads to the resistance of phytopathogens. A promising alternative can be the use of nanoparticles (NPs) as sterilizing agents.[12,13]

MATERIALS & METHODS

The Stock Solution of 1% HA, 1% Carageenan, 1% Gelatin are taken. The polysaccharides were mixed to a homogenous solution. Rutin doped copper oxide nanoparticles was added to the solution for the test group. Homogenous Solution transferred to six well plate, 100ml of crosslinking agent TPP (15%). Plates stored at -20 ^{0}C for 24 hours, followed by -80 ^{0}C overnight. Lyophilised for 24 hours and stored in dry Condition.

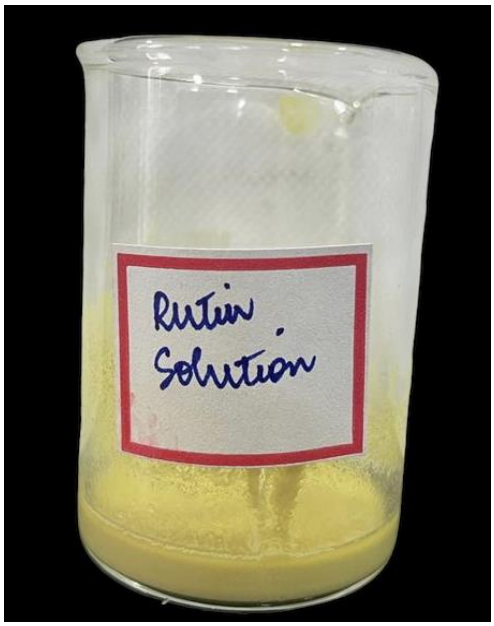


Fig 1- Rutin solution

Fig 2 -Copper nitrate solution

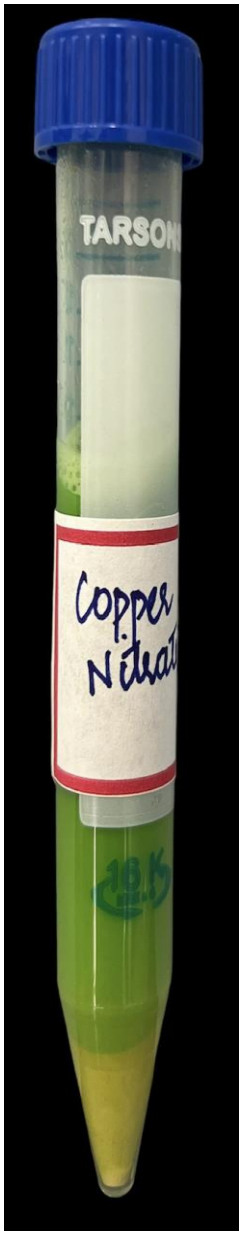
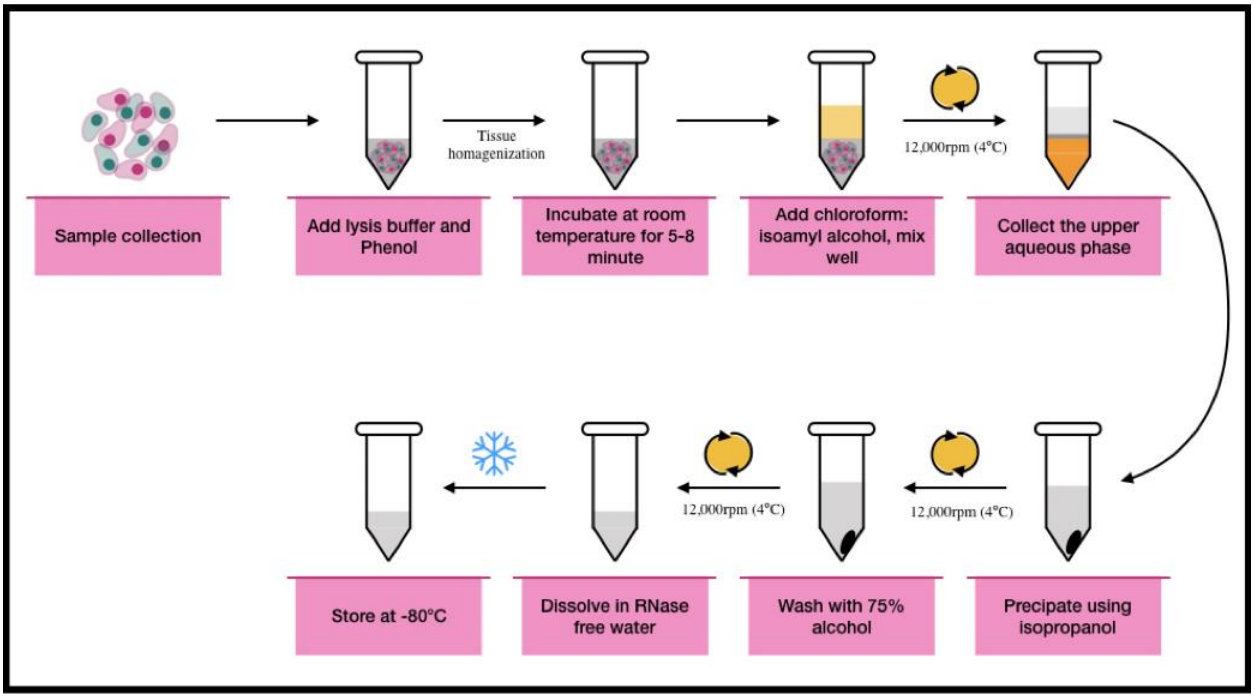


Fig 3 - Rutin doped copper oxide nanoparticles solution

RESULTS AND DISCUSSION:

The Copper Oxide coated scaffold showed that the osteogenic gene expression of osteocalcin and collagen 1 in the copper oxide containing group was highest compared with that in the

control groups. The results indicated that the copper coating facilitated cell adhesion and enhanced growth and osteogenic inductive capacity.



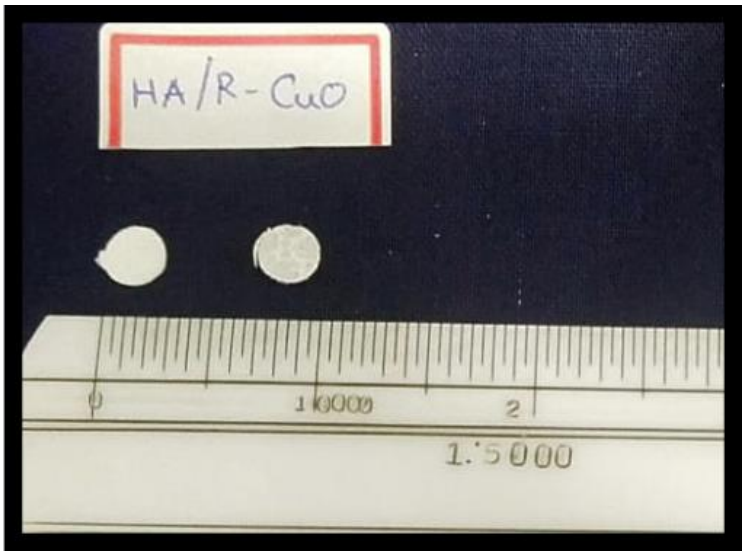
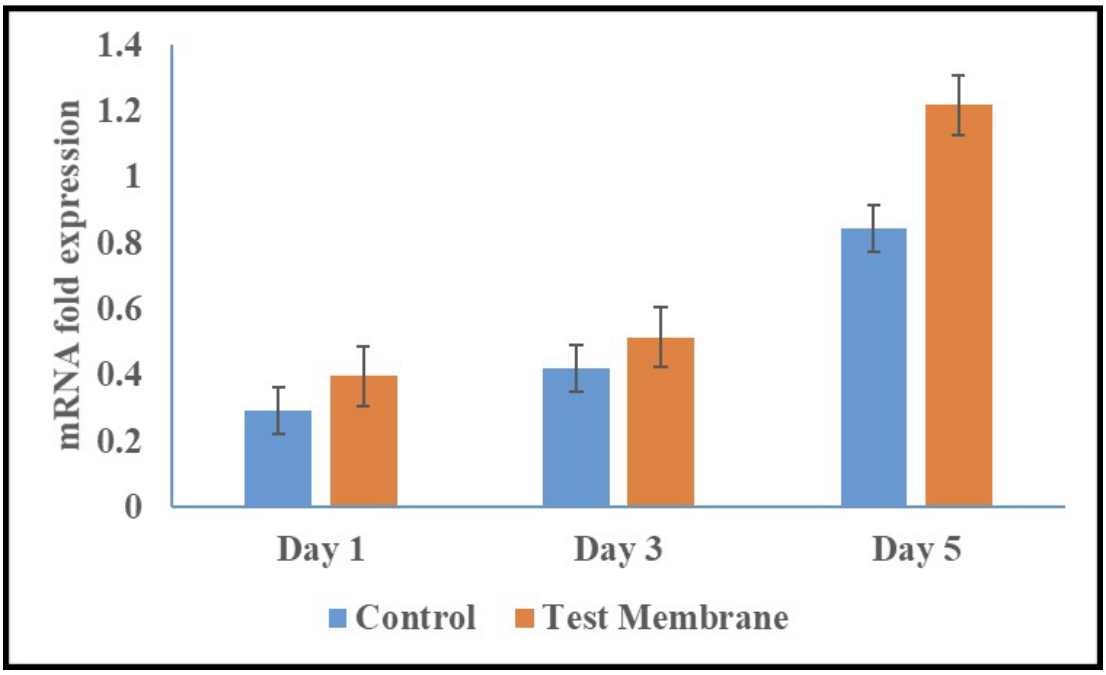
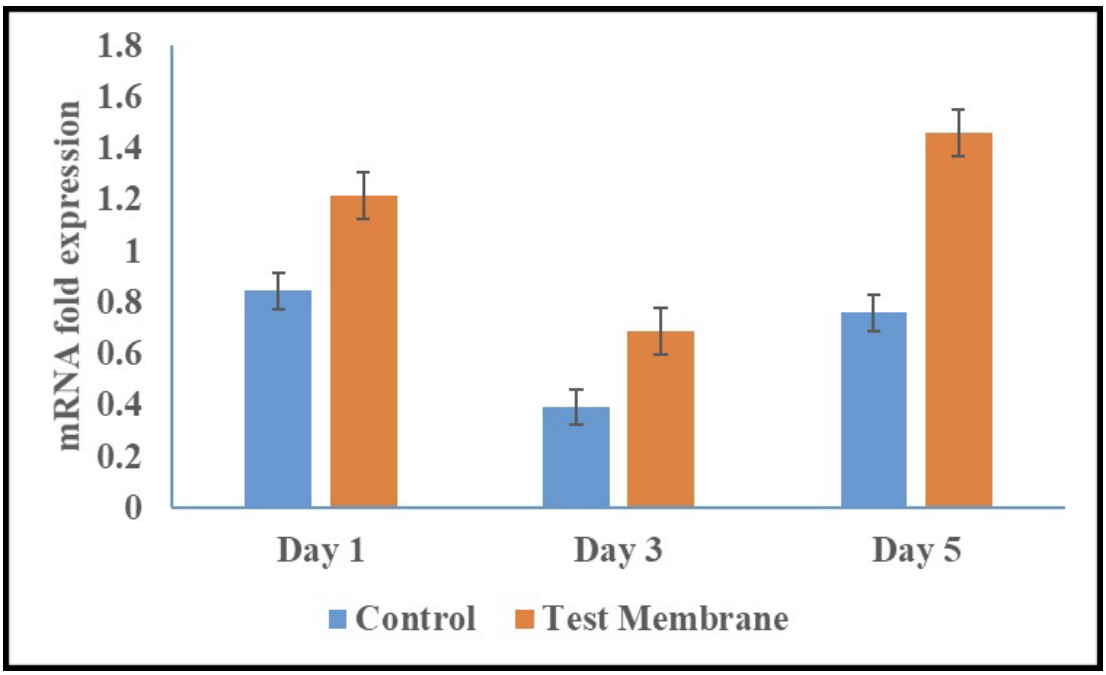


Fig 4- Copper oxide coated scaffold

GROUP	DAY 1 (ng/µL)	DAY 3 (ng/µL)	DAY 5 (ng/µL)
CONTROL	5.22	4.59	8.64
CuO	13.59	5.85	0.63



Graph 1:Osteocalcin



Graph 2: Collagen

From the graphs below we can understand Copper oxide coated scaffold showed that the osteogenic gene expression of osteocalcin and collagen 1 in the copper oxide containing group was highest compared with that in the control groups.The results indicated that the Cu coatings facilitated cell adhesion and enhanced growth and osteogenic inductive capacity.From the previous articles copper is essential for the metabolism of the skeleton, and copper deficiency leads to bone abnormalities[14].Copper deficiency can reduce the activities of monoamine oxidase and LOX, leading to increases in the solubility of bone collagen and damage to the connection of peptide chains, which in turn damages the stability of bone collagen and reduces bone strength.Copper can promote the adhesion and proliferation of osteoblasts as well as osteogenic differentiation of MSCs, however the underlying mechanisms are not fully understood.In addition to promoting osteogenic differentiation, some studies have found that copper also promotes chondrogenic proliferation and differentiation.

Copper-based Nanoparticles are particularly considered as a promising antimicrobial agent against a wide spectrum of pathogens. Being plant protection agents, Cu-based NPs displayed a greater fungicidal efficacy than a commercial preparation based on Cu(OH)2 and ionic forms of

copper[15] It is familiar that copper-based NPs can have a positive effect on plant development both during traditional seed germination and in tissue culture. Nanoparticles are used as an elicitor for the synthesis of bioactive compounds, because they can affect the secondary metabolism in plants, as well as in culture systems. Metal Nanoparticles are generally believed to reduce stress by increasing photosynthetic activity, the formation of phenolates, which can act as antioxidants in cases of excessive production of ROS and by the up-regulation of stress-response genes encoding pathogenesis-related (PR) proteins and antioxidant enzymes, transcription factors (TFs), associated with water stress late embryogenesis abundant (LEA) proteins, dehydrins, and aquaporins. Iron, copper, cobalt, and zinc oxide nanoparticles can improve the relative water content, drought tolerance index, biomass reduction rate, and positively regulated resistance gene expression in drought-exposed soybean leaves and roots[16]. The combined use of elevated CO2- and Ni-based NPs improved growth and photosynthesis and mitigated nickel-induced oxidative stress in wheat.

Among the other major vital metals, copper is noteworthy due to its significant physiological role in enzymatic reactions and electron transfer associated with energy generation. Copper nanoparticles (CuNPs) are biocompatible and possess enhanced antioxidant and antidiabetic activity that are advantageous for wound healing. [17]It can stimulate the migration and proliferation of endothelial cells and can also stimulate angiogenesis. Furthermore, copper treated wounds exhibited enhanced tissue regeneration, thereby facilitating the healing process. Therefore, copper is considered as a promising metal to be incorporated in nanoscaffolds for bone tissue regeneration applications.[18]

The earlier studies incorporated copper into chitosan scaffolds to enhance bone tissue regeneration. Initially, the chitosan sponges were prepared by dissolving chitosan in a 0.3 M acetate buffer at a concentration of 2% w/v which was then lyophilized. Furthermore, copper-chitosan scaffolds were fabricated by reacting the chitosan solution with a 0.625 mM copper solution for 4 h. Pores 104 ± 5 µm in diameter in the chitosan scaffolds exhibited an interconnecting network, while the incorporation of copper in the scaffolds showed no change in such micro-structural aspects. Undeviating mineralized bone tissue formation was evident 4 weeks after implantation of copper-chitosan scaffolds into critical-sized calvarial defects (5 mm

diameter) in Fischer 344 male rats.[19] This characterization enriched that the osteogenesis might be attributed to the macroporous nature of the scaffolds that facilitated the infiltration of cells to migrate to the site of injury. Furthermore, the walls of the porous matrix provided support for the attachment and anchoring of cells. Larger pore sizes permitted space for adequate space for cell growth, proliferation, differentiation, and angiogenesis, resulting in better bone tissue development.

Functional HREs encode proteins involved in angiogenesis (VEGF, endothelin-1), maturation of red blood cells (erythropoietin and transferrin), energy metabolism (glucose transporter 1 and 3), and cell proliferation and viability (insulin-like growth factor 2, p21). This primes to an increase in the secretion of VEGF and BMP-2 proteins. Upon transplantation into critical-sized calvarial defects in rats, the GO-Cu-coated CPC scaffolds noteworthily promoted both angiogenesis and osteogenesis. Phagocytosis of the GO-Cu nanocomposites by multinucleated giant cells showed significant vascularized bone regeneration[20] Nanocomposites with a dual role of bone tissue regeneration as well as antimicrobial activity are more preferred biomaterials for orthopedics as they can promote bone healing as well as resist biofilm associated implant failure. Incorporated copper within mesoporous bioactive glasses (MBGs) to facilitate both osteogenesis and angiogenesis apart from inhibiting bacterial growth.

Bone tissue has a dynamic nature and it demonstrates the unique property of the continuous self-repair and renewal throughout the life. Nevertheless, the regeneration of the damaged bone tissue is a significant challenge in the medical field. The extensive bone loss in tumors, infections, critical size bone fractures, and metabolic bone diseases lead to impaired bone healing. In these situations, the surgical intervention and bone substitute for promoting bone regeneration are necessary. Bone substitutes should act as a proper template for the ingrowth of the new bone, and this means that they should have the osteoconductive property. Also, to support the cell migration and proliferation, it should be osteoinductive and it should be capable of inducting the stem cells/preosteocytes to osteogenic differentiation. Besides, such an ideal construct should be biocompatible, bioresorbable, and it should have proper osteointegration with the host tissue.[21]

Bioactive factors are the significant part of the bone tissue engineering systems for native-mimicking of the bone microenvironment and events during the bone self-healing. Complex molecular signaling pathways and a large number of the cytokines, hormones, and growth factors regulate these biological events . Among them, the growth factors (GFs) are the most potent and widely studied induction factors for bone reconstruction. These molecules have an intrinsically critical role in bone healing[22] . Nevertheless, GFs have some known drawbacks including the high cost, difficulty in handling, immunogenicity, and a short half-life. The other molecules that are able to promote bone formation by activating the particular signaling pathways related to the bone renewal, can be a good replacement for GFs.

An increasing number of small molecules and drugs with the organic nature, also molecules such as the bioceramics and inorganic nanoparticles are suitable candidates for scaffold additives to facilitate bone repair. Some of them are capable of induce the osteogenic differentiation of progenitor cells and they lead to osteogenesis. Some others could improve the osteoconductivity, or they could enhance the osteointegrity by increasing the bone density[23]. Several small molecules can upregulate the VEGF expression, which consequently promote angiogenesis. Some of them can reduce the osteoclast differentiation to prevent the bone resorption, and enhance the osteoblast differentiation to increase the bone formation.

CuO NPs had pronounced concentration-dependent antifungal effects on phytopathogens in culture. This is consistent with the results of a number of previous studies, where the authors demonstrated the suppression of the phytopathogenic fungi growth such as Botrytis cinerea, F. oxysporum, Aspergillus sp., A. alternata, Fusicladium oleagineum, and Colletotrichum sp. Both the diffusion of copper ions, which is an antimicrobial agent, and specific nanotoxic effects, such as the induction of oxidative stress or damage to the cell membrane, are considered as possible mechanisms.[24]

Copper is essential for the metabolism pf the skeleton, and copper deficiency leads to bone abnormalities. Copper deficiency can reduce the activities of monoamine oxidase and LOX, leading to increase in the solubility of bone collagen and damage to the connection of peptide chains, which in turn damages the stability of bone collagen and reduces bone strength. Copper

can promote the adhesion and proliferation of osteoblasts as well as osteogenic differentiation of MSC, however the underlying mechanisms are not fully understandable [25]. Additionally, promoting osteogenic differentiation, some studies have identified that copper can also promote Chondrogenic Proliferation and differentiation.

CONCLUSION:

The Green Synthesised (Rutin) copper Oxide Nanoparticles impregnated Scaffolds has Osteoinductive Potential that Promotes the differentiation of Stem Cells. Further Studies are required implementing more Clinically relevant large animal modes to fully elucidate the effect of the Copper biomaterials on in situ bone and cartilage regeneration.

REFERNCES:

1. Patel JK, Pathak YV. Emerging Technologies for Nanoparticle Manufacturing. Springer Nature; 2021.
2. Grodetskaya TA, Evlakov PM, Fedorova OA, Mikhin VI, Zakharova OV, Kolesnikov EA, et al. Influence of Copper Oxide Nanoparticles on Gene Expression of Birch Clones In Vitro under Stress Caused by Phytopathogens. Nanomaterials (Basel) [Internet] 2022;12. Available from: http://dx.doi.org/10.3390/nano12050864
3. Antonio-Pérez A, Durán-Armenta LF, Pérez-Loredo MG, Torres-Huerta AL. Biosynthesis of Copper Nanoparticles with Medicinal Plants Extracts: From Extraction Methods to Applications. Micromachines 2023;14:1882.
4. Thomas AA, Varghese RM, S Rajeshkumar. Green synthesis of copper nanoparticles using green tea and neem formulation and assessment if its antimicrobial effects. Indian J. Forensic Med. Toxicol. 2022;16:119–24.
5. Fathi-Achachelouei M, Knopf-Marques H, Ribeiro da Silva CE, Barthès J, Bat E, Tezcaner A, et al. Use of nanoparticles in tissue engineering and regenerative medicine. Front. Bioeng. Biotechnol. 2019;7:113.
6. View of Green Synthesis of Silver Nanoparticles from Aloe Vera and Neem Leaf Extract and their Cytotoxic Effect evaluation [Internet]. [cited 2025 Jan 25];Available from: https://doi.org/10.47750/jptcp.2023.30.12.020
7. Abirami S, George AM, Rajeshkumar S. Efficacy of biosynthetically developed selenium nanoparticles using plant extracts of clove and cardamom. Int. J. of Health Sci. 2022;6:9799–6811.

8. Rajeshkumar S, Santhoshkumar J, Vanaja M, Sivaperumal P, Ponnanikajamideen M, Ali D, et al. Evaluation of Zebrafish Toxicology and Biomedical Potential of Aeromonas hydrophila Mediated Copper Sulfide Nanoparticles. Oxidative Medicine and Cellular Longevity 2022;2022:7969825.

9. Kamala Devi S, Rajasekar A, Rajeshkumar S. Anticariogenic Activity of Copper Nanoparticles Synthesized Using Blue Tea. J. Pharm. Res. Int. 2021;278–89.

10. Johnson J, Shanmugam R, Lakshmi T. A review on plant-mediated selenium nanoparticles and its applications. J Popul Ther Clin Pharmacol 2022;28:e29–40.

11. View of A Review on plant mediated selenium nanoparticles and its applications [Internet]. [cited 2025 Jan 25];Available from: https://doi.org/10.47750/jptcp.2022.870

12. Yuan Z, Li Y, He Y, Qian K, Zhang Y. Differential Analysis of Three Copper-Based Nanomaterials with Different Morphologies to Suppress Alternaria alternata and Safety Evaluation. Int. J. Mol. Sci. 2023;24:9673.

13. Liu M, Mo Y, Dong Z, Yang H, Lin B, Li Y, et al. Antibacterial activity of zinc oxide nanoparticles against Shewanella putrefaciens and its application in preservation of large yellow croaker (Pseudosciaena crocea). Food Res Int 2025;201:115642.

14. Ghosh S, Webster TJ. Metallic Nanoscaffolds as Osteogenic Promoters: Advances, Challenges and Scope. Metals 2021;11:1356.

15. Velumani P, Palani N, Antalin Casmie A, Senthilvel R, Parthasarthy V. Cellular and chromosomal interaction of bio-synthesized copper oxide nanoparticles - Induced nano-cytotoxicity and genotoxicity. Toxicol In Vitro 2024;104:106000.

16. Eldera SS, Alkhtaby LA, Al-Wafi R, El-Nour MA. Biosynthesis of Zinc Oxide Nanoparticles by Origanum majorana Aqueous Leaves Extracts, Characterization and Evaluated Against to Schistosoma haematobium. J Biomed Mater Res B Appl Biomater 2025;113:e35538.

17. Varghese R, Maliael M, Subramanian A. Antibacterial activity of nanoparticle-coated orthodontic archwires: A systematic review. J. Int. Oral Health 2023;15:1.

18. Singh AK, Jeevitha M, Rajeshkumar S, Jayaraman S. Green Synthesis and Antioxidant Activity of Silver Nanoparticles Synthesized Using Ficus benghalensis. J. Pharm. Res. Int. 2021;114–23.

19. Purushothaman E, Shanmugavadivu A, Balagangadharan K, Lekhavadhani S, Saranya I, Babu S, et al. Osteogenic potential of esculetin-loaded chitosan nanoparticles in microporous alginate/polyvinyl alcohol scaffolds for bone tissue engineering. Int J Biol Macromol 2025;286:138518.

20. Jeong H, Subramanian K, Lee JB, Byun H, Shin H, Yun JH. Anti-inflammatory and osteoconductive multi-functional nanoparticles for the regeneration of an inflamed alveolar bone defect. Biomater Sci [Internet] 2025;Available from: http://dx.doi.org/10.1039/d4bm01280a

21. Gao C, Chen Y, Wen X, Han R, Qin Y, Li S, et al. Plant-derived exosome-like nanoparticles in tissue repair and regeneration. J Mater Chem B [Internet] 2025;Available from: http://dx.doi.org/10.1039/d4tb02394c

22. Bhaskar B, Rao PS, Kasoju N, Nagarjuna V, Baadhe RR. Biomaterials in Tissue Engineering and Regenerative Medicine: From Basic Concepts to State of the Art Approaches. Springer Nature; 2021.

23. Stellpflug A, Caron J, Fasciano S, Wang B, Wang S. Bone-derived nanoparticles (BNPs) enhance osteogenic differentiation Notch signaling. Nanoscale Adv [Internet] 2024;Available from: http://dx.doi.org/10.1039/d4na00797b

24. Husain S, Sundari S, Jain RK, Kumar SR. Green synthesis of Terminalia Arjuna-mediated Hydroxyapatite Nanoparticles, its morphological assessment and evaluation of cytotoxic and antioxidant properties: An in-vitro study. J. Clin. Diagn. Res. [Internet] 2023;Available from: https://www.jcdr.net//article_fulltext.asp?issn=0973-709x&year=2023&volume=17&issue=3&page=FC06&issn=0973-709x&id=17659

25. Inamuddin, Altalhi T. Green Sustainable Process for Chemical and Environmental Engineering and Science: Biomedical Applications of Green Composites. Elsevier; 2023.