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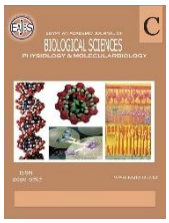

Enhancement of Rat Bone Marrow Stem Cells Using Nanomaterials

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ABSTRACT

Bone marrow stem cells (BMSCs) exhibit considerable potential for regenerative medicine; nevertheless, their clinical utilization is constrained by invasive extraction techniques and suboptimal cell yields. This study examines the potential of nanomaterials to enhance the viability and functionality of BMSCs, offering a non-invasive and secure alternative to conventional methods. We extracted and cultured bone marrow stem cells (BMSCs) from adult male Western Albino rats and assessed their responsiveness to selenium nanoparticles (SeNPs) and melatonin by cell viability assays (MTT). The results indicated that SeNPs markedly enhanced BMSC viability ($p < 0.001$) relative to control groups, with peak effects noted at 48–72 hours. A morphological study verified the effective proliferation and differentiation of BMSCs, achieving 95–100% confluence within 10 days. These data indicate that nanomaterials, especially SeNPs, can improve BMSC functionality by altering the stem cell microenvironment. Further study is required to clarify the underlying principles and enhance nanoparticle formulations for therapeutic applications. This study underscores the capacity of nanotechnology to enhance stem cell-based therapeutics for bone repair and other regenerative applications.

INTRODUCTION

Bone marrow stem cells (BMSCs) have attracted a great deal of attention as a new means for cell-based therapies, with various experiments in cell transplantation and gene manipulation for the regeneration of lost bone tissues due to degeneration (Du *et al.,* 2020). However, the harvest of BMSCs from patients is invasive, and the number of cells obtained is limited. The use of induced pluripotent stem (iPS) cells appears promising, but iPS cells are obtained through genetic manipulations and have risks of tumorigenesis. For these reasons, a non-invasive and safe enhancement method of BMSCs is desired, with an emphasis on the application of nanomaterials (Yanada, 2025; Tammam *et al.,* 2023).

Stem cells are primitive cells that have the potential to self-renew and develop into different specialized functional cells. They can also maintain an undifferentiated state to become self-renewed daughter cells, which may develop into a variety of specialized functional cells to meet the physiological requirements of the organism.

Stem cells can be classified according to their developmental stage into two broad types, embryonic stem cells (ESCs) and somatic stem cells (SSCs). Embryonic stem cells (ESCs) are derived from the inner cell mass of blastocysts that are pre-implantation embryos (Wei *et al.,* 2017). Induced pluripotent stem cells (iPSCs) are produced from somatic cells by genetically reprogramming them to an ESCs-like state. They have an unlimited source and can be derived from any individual. ESCs and iPSCs are pluripotent stem cells with great differentiation potential and infinite self-renewal capacity. However, ESCs are difficult to obtain and controversial in ethics. iPSCs are produced through tailored modification of exogenous genes or small chemical agents. These exogenous genes and agents are usually DNA viruses, which can easily lead to mutational damage in the host genome. SSCs, on the other hand, derived from adult tissues, are more accessible but less potent than ESCs and iPSCs. Adult stem cells usually reside within tissues and are involved in tissue homeostasis. In addition to SSCs, mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs), as well as neural stem cells (NSCs), have become attractive stem cell sources for tissue regeneration.

Stem cells hold great promise for regenerative medicine, as they have potential applications in cell therapy and construction of tissue-engineered grafts. The clinical application of stem cells, especially in cell therapy and tissue engineering, depends on the regulation and control of cell differentiation into specific cell types. A variety of biological and synthetic materials combined with growth factors have been investigated to mimic stem cell microenvironments to direct stem cell differentiation. Embryonic stem cells have been the model of pluripotent stem cells, and there is a growing interest in controlling their differentiation. Great efforts have been made to manipulate the differentiation of stem cells into numerous types of cells, such as osteoblast cells, neurocytes, adipocytes, and cardiomyocytes. However, the low differentiation efficiency and success rate still limit the development of stem cell differentiation for stem cell therapy. For example, the percentage of differentiated side population cells with cardiomyocyte-like structure after 20 days in cardiomyocyte differentiation was only 9.3%. Additionally, undifferentiated ESCs after implantation increase the risk of teratoma. In order to safely use ESCs in cell-based therapies, it is important to allow committed differentiation of ESCs prior to implantation. Thus, there is an urgent need to develop strategies to improve the efficiency of direct differentiation of stem cells into specified cell types.Thus, there is an urgent need to develop effective strategies to improve the efficiency of directed differentiation of stem cells into specified cell types (Farokhi *et al.,* 2021).

Bone marrow stem cells (BMSCs) are multipotent stem cells that can be harvested from mammals and differentiate into bones, cartilage, and fat. BMSCs are used as a cell source for tissue engineering (Rossi *et al.,* 2023). For tissue engineering, BMSCs must expand in vitro in either a feeder-cell-based or a feeder-free system. With many commercially available BMSCs, feeder-free systems are more favorable for cancer patients receiving cellular therapy because these patients are typically treated with a chemical that attacks rapidly dividing cells such as feeder-cell-based stem cells (Kalaiselvan *et al.,* 2024). BMSCs are known to preferentially adhere to culture plates; thus, culture plates functionalized with extracellular matrix (ECM) proteins such as collagen are usually used to culture BMSCs in a feeder-free manner. If tissue engineering is to be accelerated, rapid proliferation of BMSCs is required. In the past two decades, various culture methods have been developed to expand BMSCs in a feeder-free manner at low cost. These include coating culture plates with either bone- or cartilage-specific polymers, fibronectin, or poly-L-ornithine or enabling cell adhesion with poly(ethylene

glycol) hydrogels. BMSCs cultured for more than 12 passages in a growth factor-containing expansion medium remained bone- or cartilage-restricted but were not multipotent. Addition of growth factors or extracellular matrix proteins failed to make these aged BMSCs regain multipotency. Nevertheless, some of these BMSCs produced cartilage when implanted into surgically induced cartilage defects (Isosaari *et al.,* 2023).

Several factors have been established to influence SC behavior, including biochemical, mechanical, and topographical cues (Metavarayuth, K.,2016). Physical cues such as EC and modulus have also been shown to play an important role in SC niche (Atcha, H.,2023). However, the effect of topographical cues on SC behavior is less established compared to biochemical and mechanical cues. A study where MSCs were seeded on nano-topographic surfaces with varying features sizes showed that nano-scaled features promoted stemness of multiple MSC types, supporting the idea that nano-scale topographical features can regulate SC behavior

Drug delivery or drug-targeting devices of less than 500 nm are generally called nanocarriers or nanoparticles. Such a nanocarrier upon injection into the vascular system affects the distribution and bioavailability of the drug in the whole body and in the target(s). Nanomaterials have attracted interest because of their volumetric, surface, and quantum effects, including high specific surface area, ultra-clipboard effect, and improved mechanical properties such as hardness, elasticity, and strength, among others, compared with their coarser counterparts (Alrashdi *et al.,* 2023; El-Khawaga *et al.,* 2023). Due to their tiny sizes and high specific surface areas, it is expected that surface properties significantly affect the behavior and phenomena of materials at the nanoscale. It is noteworthy that any measurement or utilization of nanomaterials will change their attributes in the highly complex physiological environment in vivo or in vitro (Ulusoy, 2023). Therefore, the consideration of surface properties is important and beneficial for the development of nanomaterials at the initial design stage. On the other hand, biomedical applications of nanomaterials mainly rely on their surface properties because surface properties such as sizes and the presence of functional groups significantly affect in vivo protein/ nanocarrier interaction and subsequent cellular uptake, distribution, and biodegradability. Nanomaterials can be modified by coating them or coupling them with natural or synthetic polymers or bioactive molecules to improve their properties. The surface functionalization of nanomaterials can broadly be approached in two ways: non-covalent and covalent approaches. Non-covalent functionalization generally includes electrostatic adsorption, hydrogen bonding, pi-pi stacking, covalent bond breaking, and van der Waals attraction. Each nanomaterial has its own advantages and drawbacks (Elmowafy *et al.,* 2023).
Nanomaterials are defined as a natural, incidental, or manufactured material containing free or agglomerated particles, in which 50% or more of the particles have one or more external dimensions (length, breadth, or height) in the size range of 1-100 nm. Nanomaterials include bulk nanostructured materials, nanoparticles, nanorods, nanostars, nanowires, carbon nanotubes, carbon black, graphene oxide, fullerene, doped diamond, nanohorn, and silica-based nanoparticles (Tanaka *et al.,* 2022). Advancements in technology have enabled precise manipulation of atoms, ions, and molecules at an individual level, facilitating their integration into nanomaterials that conform to specific sizes or quantifiable ranges. This concept was notably highlighted by Feynman in his 1960 lecture, “There’s plenty of room at the bottom,” where he proposed that the ability to induce chemical reactions at the atomic level could fundamentally transform chemical technology through careful observation and handling of singular atoms. This idea laid the groundwork for Eric Drexler’s promotion of "molecular nanotechnology" (MNT), effectively

founding the area of molecular nanoscale engineering. The versatility of nanomaterials is evidenced by their diverse applications, which include use in cosmetics, as catalysts, in quantum dots, in anti-soiling coatings, as photocatalysts, as contrast agents for luminescence imaging, in drug delivery systems, as tags for in vitro assays, in liquid crystal display interfaces, in nano-interconnects and through-silicon vias (TSVs), as electrodes for batteries and Hargraves cells, andas wiring materials utilized in integrated circuits (Anjaneyulu *et al.,* 2024).

Nanomaterials have garnered considerable attention across multiple fields of research, including material sciences, pharmacology, and biology, owing to their superior mechanical properties and the effects associated with their volume, surface, and quantum characteristics. For instance, the compressive and flexural strengths in both compressive and tensile tests of ordinary Portland cement mortars that include de-wetted nano-SiO_2 or nano-Fe_2O_3 were found to be superior to those of a control group. Additionally, nano-Al_2O_3 ceramics demonstrated enhanced flexural strengths when compared to their microscale monolithic alumina ceramic equivalents (Gohar *et al.,* 2024). When dispersed in common materials, nano-sized pin industry particles refine the grains of the material to an extent that they are then able to form intra- or inter-granular structures, improving the arrangements of the grain boundaries and, in turn, promoting ultimate and flexural strength. In biological applications, surface properties are considered a main reason why the material behaves differently in the nanoscale region. Altering surface properties or functionalization of the nanomaterials enables the improvement and/or addition of properties useful in medical applications (Griffin *et al.,* 2016; Ulusoy, 2023). The modification of the nanomaterial surface can be achieved through two different approaches: non-covalent adsorption and covalent attachment. The covalent attachment of ligands to the nanomaterial surface is performed using linker molecules. The advantage with this approach is that several ligands can be attached to individual nanoparticles, resulting in multiple functionalization of a nanoconstruct (Guan *et al.,* 2023). The recent efforts have included the preparation of sensitive binding using pH-sensitivity and thermo-sensitivity to develop a nanoplatform for controlled drug release. Each of the various nanomaterials has their own advantages and drawbacks. Ceramics are in general regarded as bioceramics, being similar to bone components and are believed to have osteoinductive ability and biocompatibility. On the other hand, practicality is often a concern, because by its lower resorptive rate or rapid dissolution, either biocompatibility or the cytotoxicity, respectively, of the nano-sized ceramic when taken up by cells is often a concern. Polymeric nanoparticles are highly bioabsorbable materials and, thus, regarded as relatively safe materials (Tanvir *et al.,* 2024).

Surface properties are the most important reason that materials behave differently at a nano-size. As the particle size decreases, large areas of particle surfaces, or surfaces that can interact with the surrounding environment, become the predominant factor that influences various properties. Hence, the modification of nanomaterials' surfaces has been conducted, which could be achieved by both non-covalent and covalent approaches (Shekhawat *et al.,* 2022). Non-covalent functionalization is the binding of surfactants, biomolecules, and polymers to the surface of nanomaterials with van der Waals or ionic interactions. Nanomaterials functionalized by non-covalent approaches have the possibility to displace surfactants and biomolecules upon contact with biological environments. On the other hand, the binding of small molecules and polymers could be covalently linked to the surface of nanomaterials through silane coupling reactions, covalent amine reactions, and other crosslinking organic reactions. Since polymers are highly bioabsorbable and regarded as relatively safe materials, they have been an attractive means of surface modification for nanomaterials (Tanaka *et al.,*

2022).

Cells in physiological condition are challenged by both soluble and insoluble stimuli from the microenvironment. Most cells either sit on the extracellular matrix (ECM) or are embedded in it, which provides both the chemical and physical properties to support cell migration, proliferation, survival, and differentiation (Dzobo & Dandara, 2023). In addition to these biochemical signals, cells in the development stage and in tissue maintain their functions not only by various soluble factors but also by nanoscale topographical patterns in vivo. Nanoscale features have caught researchers' attention. These nanoscale structures or features consist of small fibers, pits, and grafts on which cells can grow and exert their function in such features that mimic the in vivo microenvironment. This nanoscale architecture promises that nanotechnology will become an important area for tissue engineering and regenerative medicine (Narayanan, 2025).

Examples of Some Properties for Some Nanoparticles:

***Silica Nanoparticles (SiO_2-NPs):** Human mesenchymal stem cells (hMSCs) can be tracked with silica nanoparticles (SiO_2-NPs). Prior research demonstrated that hMSCs were able to survive SiO_2-NP absorption without developing genotoxic stress, cell death, or impaired capacity for proliferation and differentiation (Popara, J., 2018).

***Selenium Nanoparticles (SeNPs):** Selenium is an essential element found in many antioxidant enzymes and proteins, where it helps protect the body by reducing oxidative stress. Compared to other forms of selenium, selenium nanoparticles (SeNPs) offer stronger biological activity, lower toxicity, and still provide all of selenium's normal physiological benefits. This makes SeNPs the most effective form of selenium (Luo L, *et al.,* 2021).

***Iron Oxide Nanoparticles (Fe_2O_3 nanoparticles):** These nanoparticles can be designed to interact with mesenchymal stem cells (MSCs), which are recognized for their therapeutic and regenerative potential. They are frequently utilized as contrast agents in magnetic resonance imaging (MRI) and drug delivery systems (Pourmadadi, M. *et al.,* 2022).

MATERIALS AND METHODS

Animal Groups:

Ten adult male Western Albino rats were used in this study. The rats that were chosen had weights ranging from 80 to 150 grams and ages spanning from 6 to 8 weeks. Before being used as experimental animals, all the rats underwent a thorough examination and were observed for a week to weed out any animals that were infectiously ill. The Faculty of Medicine Ain Shams Medical Research Institute Animal Facility (MASRI-animal) is home to rats that were purchased and kept. They were provided with food and water on an unlimited basis, allowing them unfettered access to these supplies, and they were kept in metal cages covered in mesh wire. The housing environment will be kept at a comfortable, well-ventilated temperature of between 20 and 23 degrees Celsius. All of the rats will be examined prior to the study starting in order to eliminate any rats that show signs of nervousness or other health problems that might affect the results of the experiment. The inclusion of only healthy rats in the study is guaranteed by this screening procedure.

Preparation of Selenium Nanoparticles:

In this study, SeNPs were synthesized by mixing 5 ml of SCAE (0.1 mM/ml) with 10 ml of sodium selenite (0.1 mM/ml) at 18°C while stirring. Two drops of vitamin C (0.1 mM) have to be added in order to encourage the production of SeNPs (Korany *et al.,* 2020). The appearance of a rich red color suggested the precise synthesis of SeNPs. The average size of the SeNPs was determined using the Zetasizer (Nano series, HT Laser, ZEN 3600, Malvern, UK). The results of the characterization of SeNPs indicated that their mean zeta potential was -27.6±0.6 mV and their size was 189.5±11.2 nm (data not shown).

Isolation, Purification and Characterization of Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSC):

Isolation of BM-MSCs: Isolation and culture of BM-MSCs were performed in the Stem Cells and Tissue Culture Unit in MASRI, Faculty of Medicine, Ain Shams University. Ten male rats of average weight, 100 gm, were used to collect the bone marrow, which was harvested from the femurs and tibia bones of 7-week-old adult male rats

Method of Isolation: The animals were anesthetized by diethyl ether. The skin of the rats covering the lower limbs was sterilized. The femur and tibia were dissected and immersed in 70% ethyl alcohol for one minute. After that, the femur and tibia were washed in a petri dish containing phosphate buffer saline (PBS) and fungizone. In order to prevent contamination, the bone marrow was removed under a laminar air flow cabinet (Nuair), and surgical gloves and sterile instruments were used throughout the isolation procedure. The rats' femur and tibial bones were further cleaned and dissected from any adherent soft tissues in a 90 mm petri dish. Phosphate buffer saline (PBS) was used twice to wash the bones to ensure that no muscles remained. Using sterile bone scissors, the epiphyses on both ends of the bones were removed. The bone marrow was flushed out from the diaphysis by inserting a syringe needle filled with 3 ml of complete culture media into one of the bone ends using a syringe. The marrow plugs were expelled from the opposite end of the bones into 15 ml Falcon tubes. The marrow plugs were then dissociated by pipetting, and the dispersed cells were centrifuged at 1800 rpm for 15 minutes at 4°C. The cell pellets were washed twice with a complete medium and centrifuged. The bone marrow cells were resuspended in 20 ml of complete medium (CM) to be sure we have single cells. Centrifugation was done to the cells with the Hettich Centrifuge, and the complete media in the Falcon tube was replaced by another clear one. A sample of 20 µl was used for a cell count using the hemocytometer.

Culturing and Incubation of BM-MSCs: In a 25 cm^2 sterile flask with isolated cells with complete medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 13% Fetal Bovine Serum (FBS), 1.5% penicillin/streptomycin mixture, and 0.05% fungizone). The flasks were then incubated at 37°C in a 5% humidified CO_2 incubator (Thermo Scientific) for 12 days. The non-adherent cell was removed after three days by aspiration using a sterile pipette. The adherent cells were then washed twice with PBS. The medium was changed every 3 days. Cells were incubated for 12 days but with daily examination by inverted microscope (ZEISS-Axiovert 100). When cultures approached 90% confluence, cells were washed twice with phosphate buffer saline (PBS) and treated with 5 ml of 0.25% trypsin/0.02% EDTA for 2 min at 37°C to detach cells from the flasks. After 2 min, complete media was added to stop the trypsin reaction, then after centrifugation (by Hettich Centrifuge) at 2500 rpm for 10 min. Complete media and trypsin were removed, and mesenchymal stem cells (BM-MSCs) were dissolved in sterile phosphate buffer saline (PBS).

This culture was called primary culture or passage (0) culture. On the 12^{th} to 14^{th} day, when the cells were approximately 80% confluent (80% of the flask substrate was covered by cell monolayers), they were subcultured. The resulting culture was referred to as passage (1) culture.

Grouping Study: Ten mice were used, and the output from each of the flasks contained 3×10^6 cells at passage 0. The cells were then divided into 10 flasks (passage 1), followed by further subculture into 20 flasks, with each flask containing approximately 1×10^6 cells (passage 2). Subsequently, the cells were divided into two groups.

Group 1: untreated cells in normal media (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 13% Fetal Bovine Serum (FBS), 1.5% penicillin/streptomycin mixture, and 0.05% fungizone)

Group 2: treated cells with normal media and SeNPs.

Counting of BM-MSCs: Trypan blue was used to test the viability of cells; this method is based on the principle that living cells don't take up certain dyes, whereas dead cells do.

Then a hemocytometer was used to count the cells by LEICA light microscope and adjusted to 1.5 × 10^6 cells/mL. BM-MSCs were identified by adhesiveness properties and morphological characteristics, including elongated and spindle or fibroblastic shapes, and then were used immediately for animal treatment. After counting the cells, 1.5 × 10^6 cells were suspended in 1.0 ml of PBS and intravenously injected in each rat with an insulin syringe

Cell Viability Assay: MSC as normal cells enhanced with nano selenium and melatonin was determined using the MTT (3-[4, 5-dimethylthiazol-2-yl]-2.5-diphenyl tetrazolium bromide). Briefly, by hemocytometer, cells were plated in 96-well tissue culture plates in a range of 10^3-10^5 cells/well in a final volume of 100 µL of medium and were allowed to attach overnight at 37°C. The MTT reagent is added (20 µL per well of 5 mg/ml MTT), and the plate is incubated for 4 h to allow for intracellular reduction of the soluble yellow MTT to the insoluble purple formazan dye. Media were removed, and 200 µL detergent reagent (DMSO) were added to each well to solubilize the formazan dye prior to measuring the absorbance of each sample in a microplate reader at 550-600 nm. Six wells were used for each group. Cell proliferation was assessed as the percentage of cell proliferation compared to MSC as control cells.

Statistical Analysis:

Python 2.7.14, the Statistical Package for the Social Sciences (SPSS, version 22.0.0.2), and Python packages (SciPy 0.19 and pandas 0.20.3) were used to analyze the data [means ± standard deviation (SD) n = 10].

RESULTS

Isolation of Mesenchymal Stem Cell:

By using an inverted microscope, mesenchymal stem cells obtained from bone marrow were investigated after being cultured in normal medium and observed at 0, 3, 7, and 10 days (Fig. 1**)** from culturing time, where it was noticed that they reached the 95-100% confluence at day 10.

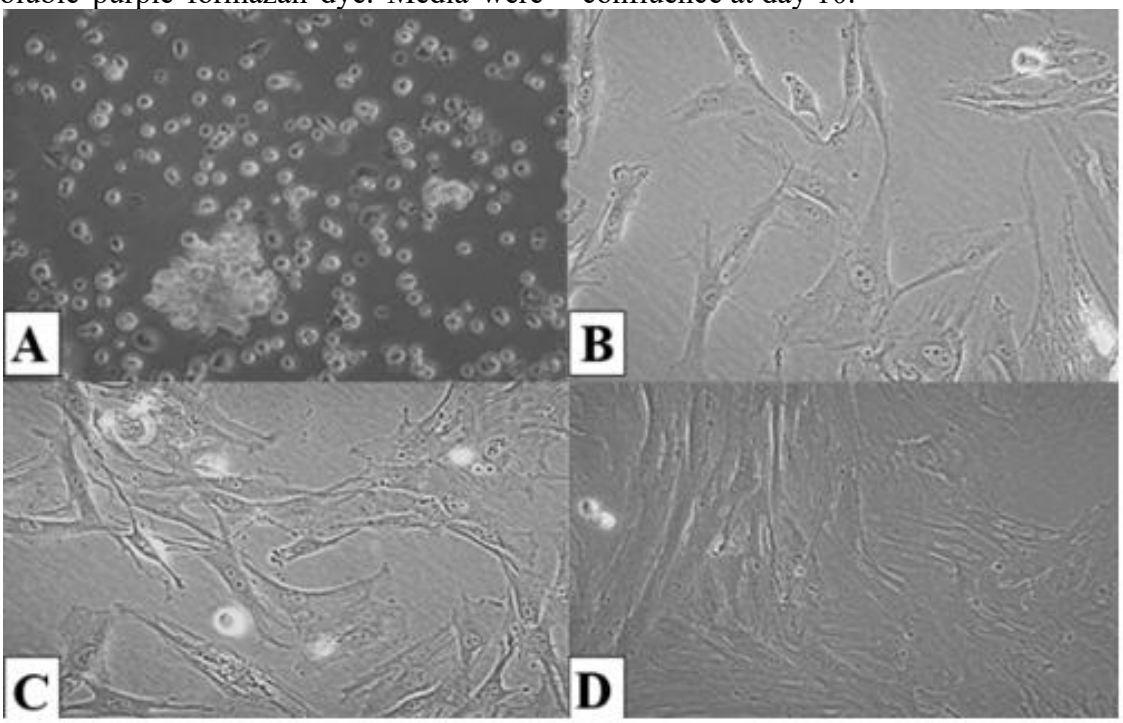

Fig. 1: Showing bone marrow mesenchymal during prefoliation in 0 passage (a) at 0 time, (b) at 3 days, (c) at 7 days, and (d) at 10 days.

Microscopic pictures of MSCs before and after they were treated with selenium nanoparticles (SeNPs) are displayed in Fig. 2. Before therapy, Fig. 2A: The morphology of the MSCs varies; some cells have irregular forms or evidence of stress, while others

appear elongated and fibroblast-like. Additionally, some of the cells are rounded, which could indicate diminished vitality or partial separation. Nevertheless, Fig. 2B (after SeNP treatment): The MSCs seem more consistent and healthier. The cells have a more spindle-like shape and are uniformly spaced over the field, giving them the fibroblast-like appearance that is typical of living MSCs. Following SeNP treatment, there are fewer rounded or detached cells, suggesting increased cell viability and proliferation. In summary, compared to untreated cells, SeNP treatment appears to improve MSC shape, adhesion, and proliferation.

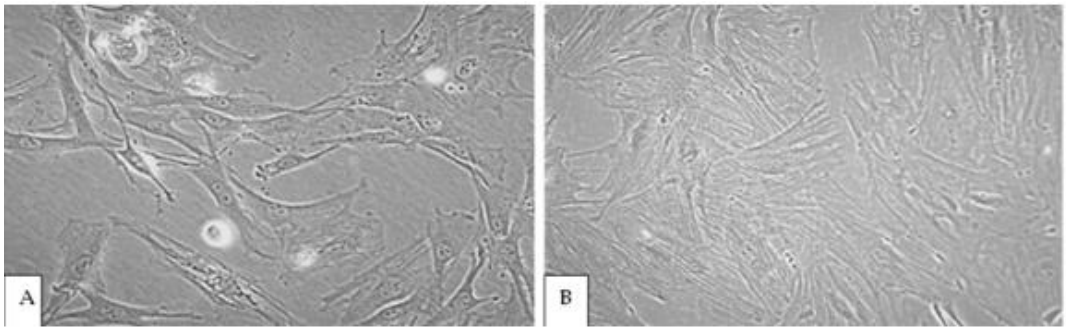

Fig. 2: Showing (A) MSCs before adding selenium nanoparticles and (B) after adding selenium nanoparticles.

Cell Viability Assay (MTT):

By using the MTT assay, it was found that there are highly significant differences in the viability between MSCs cultured in normal media, and MSCs cultured in the nano selenium ($p<0.001$), and co-culture media ($p<0.001$). By comparing the cell viability at the four-time intervals regarding the non-normally distributed variables, there was highly significant difference between the four groups (0-time (MSCs as control), 48, and 72 hrs). The data in the two box plots in Figure 3, demonstrate that the cell viability is inversely proportional to the increase of time since, after 96 hrs, the cells show the lowest value.

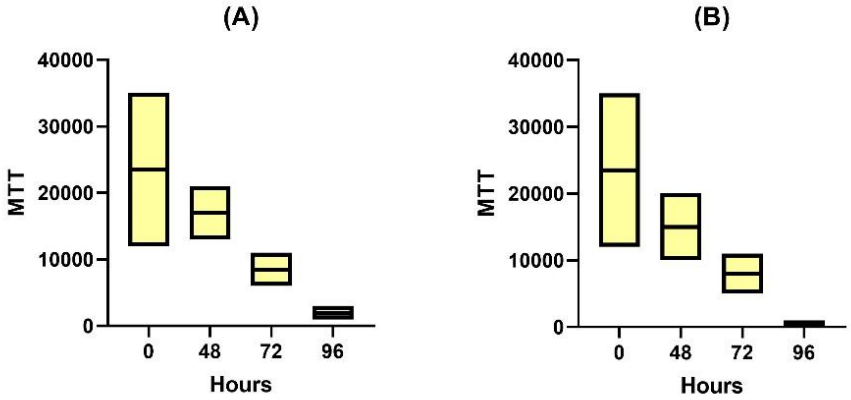

Fig. 3: MTT box plot with different times explains the distinction between (A) untreated and (B) nano selenium in enhancing bone marrow MSCs. (means appear of abnormal value).

DISCUSSION

Although nanomaterials can enhance the osteogenic differentiation of stem cells, the exact mechanisms are still not completely understood. Studies with an emphasis on the effects of nanomaterials on the proliferation and differentiation of stem cells, as well as the mechanisms responsible for such effects, are necessary. Nanomaterials applied to stem cell research should consider the cell type used in potential future clinical applications (Feng *et al.,* 2023; Du *et al.,* 2021).

Nanomaterials have received enormous attention in bone tissue engineering and stem cell research fields. Some pursue nanostructured scaffolds made from biocompatible and nanomaterial-bridged strategies to deliver stem cells for bone regeneration (Farjaminejad *et al.,* 2024). Some strategies utilize nanomaterials to assist stem cells to promote their proliferation and osteogenic differentiation. Though the synergistic effects of nanomaterials on the biological responses of stem cells are widely studied, the detailed mechanisms might be different for different sieved and shaped nanomaterials (Wu *et al.,* 2021). The pro-osteogenic potential of selected nanomaterial-coated substrates on rBMSCs was investigated in this study. These 6 coatings promoted the growth and differentiation of rBMSCs with different effectiveness, and further characterization analyses indicated that this enhancement was not solely determined by surface hydrophilicity. Hydroxylated and carboxylated nanomaterials could capture biomolecules, which activated downstream signaling pathways, enhancing the upregulation of osteogenesis-related genes. Further exploration is warranted to bridge the gap in knowledge about how the surface chemistry of C60 nanomaterials influences their protein corona (Chen *et al.,* 2022). Then, how this alteration, in turn, affects the interactions of the spike proteins with the cell membrane and the subsequent routes of entry (we predict that larger, more hydrophobic nanoparticles penetrate the cells through a different mechanism, e.g., cytoplasmic membrane shedding).

Natural bone regeneration requires coupling both osteogenesis and angiogenesis. The commitment of BMSCs to the osteogenic lineage as well as ECSs to the endothelial lineage must be enhanced simultaneously to enable bone regeneration. Therefore, tissue engineering strategies to construct vascularized scaffolds potentially revolutionize the treatment of critical-size bone defects, a challenging issue in orthopedics. Although ingenious, 3D bioprinting techniques and expensive biomolecular scaffolds have limited online applications due to the reliance on expensive instruments or bio-inks and challenges of preservation and storage. Calcium phosphate biomaterials have been demonstrated, but their rapid degradation limits their applications, which tend to be combined with biodegradable scaffolds (Li *et al.,* 2025).

During the natural healing process of bone injury or defect, successful bone regeneration requires the coordinated and coupled regulation of bone formation and blood vessel formation, which is termed osteoangiogenesis. As the key cellular participants, bone mesenchymal stem cells (BMSCs) and endothelial cells (ECSs) have undergone osteogenic or angiogenic differentiation, respectively. To promote bone regeneration, extensive research has focused on enhancing osteogenic differentiation and angiogenic differentiation and their interplay of BMSCs and ECSs **(**Du *et al.,* 2021; Jang & Yoon, 2024). However, existing dual-functional biomaterials are complex in design and preparation compared to single-component nanomaterials, which limits their wider application in this field.

Study Limitations:

Although this work shows that selenium nanoparticles (SeNPs) can improve the viability and functionality of bone marrow stem cells (BMSCs), it is important to recognize that there are a number of limitations. First, the study was carried out with rat BMSCs, which might not accurately mimic the activity of human BMSCs. As a result, the results may not be directly applicable to clinical settings. Second, the long-term effects of SeNPs on BMSC proliferation, differentiation, and possible toxicity were not investigated because the study mainly concentrated on short-term effects (up to 72 hours). Further molecular and genetic research are necessary because the mechanisms behind the reported increases were not fully explored. Additionally, the intricate in vivo milieu may not be accurately replicated in the in vitro setting, which could affect the activity of stem cells.

Declarations:

Ethical Approval: The practical part was achieved in the Faculty of Medicine at Ain Shams Medical Research Institute (MASRI). Western Albino rats were used in in vivo animal research to assess the impact of bone marrow mesenchymal stem cells (BM-MSCs) and learn more about the characteristics of stem cells and various nanoparticles that promote stem cells.

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Competing interests: The author declares no conflict of interest of any kind.

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REFERENCES

Alrashdi, B. M., Fehaid, A., Kassab, R. B., Rizk, S., Habotta, O. A., & Abdel Moneim, A. E. (2023). Biosynthesized Selenium Nanoparticles Using Epigallocatechin Gallate Protect against Pentylenetetrazole- Induced Acute Epileptic Seizures in Mice via Antioxidative, Anti-Inflammatory, and Anti-Apoptotic Activities. *Biomedicines*,*11*(7), 1955.https:// doi. org/10.3390/biomedicines11071955

Anjaneyulu, B., Tiwari, A., Chauhan, V., Bhathiwal, A. S., Ramakrishna, D. S., Praveen, P. L. (2024). Exploring versatility: Investigating nanomaterials applications in relation to polymorphism. *Journal of Molecular Structure,* 1318, 139205. doi: 10.1016/j.molstruc.2024.139205

Atcha, H., Choi, Y. S., Chaudhuri, O., Engler, A. J. (2023). Getting physical: Material mechanics is an intrinsic cell cue. In *Cell Stem Cell*. 30(6), pp. 750–765. doi: 10.1016/j.stem.2023.05.003

Chen, T., Jinno, Y., Atsuta, I., Tsuchiya, A., Stocchero, M., Bressan, E., Ayukawa, Y. (2022). Current surface modification strategies to improve the binding efficiency of emerging biomaterial polyetheretherketone (PEEK) with bone and soft tissue: A literature review. *Journal of prosthodontic research,* 67(3), pp. 337-347. doi: 10. 2186/jpr.JPR_D_ 22_00138

Du, Z., Feng, X., Cao, G., She, Z., Tan, R., Aifantis, K. E., Zhang, R., Li, X. (2021). The effect of carbon nanotubes on osteogenic functions of adipose-derived mesenchymal stem cells in vitro and bone formation in vivo compared with that of nano-hydroxyapatite and the possible mechanism. *Bioactive Materials*, 6(2), pp. 333–345. doi: 10.1016/j. bioactmat.2020.08.015

Dzobo, K., Dandara, C. (2023). The Extracellular Matrix: Its Composition, Function, Remodeling, and Role in Tumorigenesis. *Biomimetics*, 8(2), 146. doi: 10. 3390/ biomimetics 8020146

El-Khawaga, A. M., Zidan, A., Abd El-Mageed, A. I. (2023). Preparation methods of different nanomaterials for various potential applications*:* A review. *Journal of Molecular Structure*, 1281, 135148. doi: 10. 1016/j.molstruc.2023.135148

Elmowafy, M., Shalaby, K., Elkomy, M. H., Alsaidan, O. A., Gomaa, H. A., Abdelgawad, M. A., Mostafa, E. M. (2023). Polymeric nanoparticles for delivery of natural bioactive agents: recent advances and challenges. *Polymers,* 15(5), 1123. doi:10.3390/ polym15051123

Farjaminejad, S., Farjaminejad, R., Garcia-Godoy, F. (2024). Nanoparticles in bone regeneration: a narrative review of current advances and future directions in tissue engineering. *Journal of Functional Biomaterials*, 15(9), 241. doi:10.3390/jfb15090241

Farokhi, M., Mottaghitalab, F., Saeb, M. R., Shojaei, S., Zarrin, N. K., Thomas, S., Ramakrishna, S. (2021). Conductive biomaterials as substrates for neural stem cells differentiation towards neuronal lineage cells. *Macromolecular Bioscience,* 21(1), 2000123. doi: 10. 1002/mabi. 202000123

Feng, P., Zhao, R., Tang, W., Yang, F., Tian, H., Peng, S., Shuai, C. (2023). Structural and functional adaptive artificial bone: materials, fabrications, and properties. *Advanced Functional Materials,* 33(23), 2214726. doi:10. 1002/adfm.202214726

Feynman, R. (1960). Engineering & Science Magazine. *California Institute of Technology, USA*.

Gohar, O., Khan, M. Z., Bibi, I., Bashir, N., Tariq, U., Bakhtiar, M., Motola, M. (2024). Nanomaterials for advanced energy applications: *Recent advancements and future trends. Materials & Design,* 241, 112930. doi:10.1016/j.matdes.2024.112930

Griffin, M., Kalaskar, D., Seifalian, A., Butler, P. (2016). An update on the Application of Nanotechnology in Bone Tissue Engineering. *The Open Orthopaedics Journal*, 10(1), pp. 836– 848. doi: 10. 2174/ 1874325001610010836

Guan, H., Harris, C., Sun, S. (2023). Metal-Ligand Interactions and Their Roles in Controlling Nanoparticle Formation and Functions. *Accounts of Chemical Research*, 56(12), pp. 1591–1601. doi: 10.1021/acs.accounts.3c00156

Isosaari, L., Vuorenpää, H., Yrjänäinen, A., Kapucu, F. E., Kelloniemi, M., Pakarinen, T.K., Narkilahti, S. (2023). Simultaneous induction of vasculature and neuronal network formation on a chip reveals a dynamic interrelationship between cell types. *Cell Communication and Signaling*, 21(1), 132. doi:10.1186/s12964-023-01159-4

Jang, H. J., Yoon, J. K. (2024). The Role of Vasculature and Angiogenic Strategies in Bone Regeneration. *Biomimetics*. 9(2), 75. doi:10.3390/ biomimetics9020075

Kalaiselvan, E., Maiti, S. K., Shivaramu, S., Banu, S. A., Sharun, K., Mohan, D., Palakkara, S., Bag, S., Sahoo, M., Ramalingam, S., Hescheler, J. (2024), Bone Marrow-Derived Mesenchymal Stem Cell-Laden Nanocomposite Scaffolds Enhance Bone Regeneration in Rabbit Critical-Size Segmental Bone Defect Model. *Journal of Functional Biomaterials*, 15(3), 66. doi:10.3390/jfb15030066

Korany, M., Mahmoud, B., Ayoub, S. M., Sakr, T. M., Ahmed, S. A. (2020). Synthesis and radiolabeling of vitamin C-stabilized selenium nanoparticles as a promising approach in diagnosis of solid tumors. *Journal of Radioanalytical and Nuclear Chemistry*, 325, pp. 237-244. doi:10. 1007/s10967-020-07195-5

Li, S., Cai, X., Guo, J., Li, X., Li, W., Liu, Y., Qi, M. (2025). Cell communication and relevant signaling pathways in osteogenesis–angiogenesis coupling. *Bone Research*,13(1), 45. doi:10. 1038/s41413-025-00417-0

Luo, L., Wang, Y., Zhang, S., Guo, L., Jia, G., Lin, W., Gao, Z., Gao, Y., Sun, T. (2021). Preparation and characterization of selenium-rich polysaccharide from Phellinus igniarius and its effects on wound healing. *Carbohydrate Polymers*, 264, 117982. doi: 10.1016/j.carbpol.2021. 117982

Metavarayuth, K., Sitasuwan, P., Zhao, X., Lin, Y., Wang, Q. (2016). Influence of Surface Topographical Cues on the Differentiation of Mesenchymal Stem

Cells in Vitro. In *ACS (American Chemical Society) Biomaterials Science and Engineering* .2(2), pp. 142 –151. doi: 10. 1021/ acsbiomaterials.5b00377

Narayanan, K. B. (2025). Nanotopographical Features of Polymeric Nanocomposite Scaffolds for Tissue Engineering and Regenerative Medicine: A Review. *Biomimetics,* 10(5), 317. doi:10.3390/ biomimetics10050317

Popara, J., Accomasso, L., Vitale, E., Gallina, C., Roggio, D., Iannuzzi, A., Raimondo, S., Rastaldo, R., Alberto, G., Catalano, F., Martra, G., Turinetto, V., Pagliaro, P., Giachino, C. (2018). Silica nanoparticles actively engage with mesenchymal stem cells in improving acute functional cardiac integration. *Nanomedicine*, 13(10), pp. 1121–1138. doi:10.2217/nnm-2017-0309

Pourmadadi, M., Rahmani, E., Shamsabadipour, A., Mahtabian, S., Ahmadi, M., Rahdar, A., Díez-Pascual, A. M. (2022). Role of Iron Oxide (Fe2O3) Nanocomposites in Advanced Biomedical Applications: A State-of-the-Art Review. *Nanomaterials*, 12(21), 3873. doi:10. 3390/nano12213873

Rossi, N., Hadad, H., Bejar-Chapa, M., Peretti, G. M., Randolph, M. A., Redmond, R. W., Guastaldi, F. P. (2023). Bone marrow stem cells with tissue-engineered scaffolds for large bone segmental defects: a systematic review. *Tissue Engineering Part B: Reviews,* 29(5), pp. 457-472. doi: 10.1089/ten.teb.2022.0213

Shekhawat, D., Vauth, M., Pezoldt, J. (2022). Size Dependent Properties of Reactive Materials. *Inorganics*, 10(4), 56. doi:10.3390/inorganics10040056

Tammam, B. M. H., Habotta, O. A., El-Khadragy, M., Abdel Moneim, A. E., Abdalla, M. S. (2023). Therapeutic role of mesenchymal stem cells and platelet-rich plasma on skin burn healing and rejuvenation: A focus on scar regulation, oxido-inflammatory stress and apoptotic mechanisms. *Heliyon*, 9(9), e19452. doi: 10.1016/ j.heliyon.2023.e19452

Tanaka, M., Izumiya, M., Haniu, H., Ueda, K., Ma, C., Ueshiba, K., Ideta, H., Sobajima, A., Uchiyama, S., Takahashi, J., Saito, N. (2022). Current Methods in the Study of Nanomaterials for Bone Regeneration. *Nanomaterials*, 12(7), 1195. doi:10.3390/nano12071195

Tanvir, M. A. H., Khaleque, M. A., Kim, G. H., Yoo, W. Y., Kim, Y. Y. (2024). The Role of Bioceramics for Bone Regeneration: History, Mechanisms, and Future Perspectives. *Biomimetics*, 9(4), 230. doi:10.3390/biomimetics 9040230

Ulusoy, U. (2023). A Review of Particle Shape Effects on Material Properties for Various Engineering Applications: From Macro to Nanoscale. *Minerals*, 13(1), 91. doi:10.3390/min13010091

Wei, M., Li, S., Le, W. (2017). Nanomaterials modulate stem cell differentiation: biological interaction and underlying mechanisms. *Journal of Nanobiotechnology*, 15, 75. doi:10. 1186/S12951-017-0310-5

Wu, D., Chang, X., Tian, J., Kang, L., Wu, Y., Liu, J., Wu, X., Huang, Y., Gao, B., Wang, H., Qiu, G., Wu, Z. (2021). Bone mesenchymal stem cells stimulation by magnetic nanoparticles and a static magnetic field: release of exosomal miR-1260a improves osteogenesis and angiogenesis. *Journal of Nanobiotechnology*, *19*(1), 209. doi:10.1186/s12951-021-00958-6

Yanada, S. (2025). Perspectives on minimally invasive treatments using magnetically labeled bone marrow mesenchymal stem cells. *Exploration of Musculoskeletal Diseases*, 3, 100792. doi: 10. 37349/ emd. 2025. 100792