
# Comparison of the Effect of Nanocrystalline Hydroxyapatite and Stem Cells in Tibial Fracture Healing (Experimental Study)

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***Presented by***


## Arzaq Sami Abdulrazzaq Moqbel

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Aden University, Yemen


***Supervisors***


## Dr/ Mohamed Abdul Magied Katamish

Professor of Oral and Maxillofacial Surgery

Faculty of Dentistry, Ain Shams University

## Dr/ Amr Amin Ghanem

Lecturer of Oral and Maxillofacial Surgery

Faculty of Dentistry, Ain Shams University


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**Ain Shams University**
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# *Dedication*

## *This work is dedicated to*

The Soul of my Grandmother

My Dear Parents the light that leads my life

My Sisters & my Brothers

My Uncle Nuhad

My Aunt Dr. Inas

My True Friends Especially Dr. Hanan & Dr. Amani

# *Aknowlegment*

I would like to express my special thanks to my great humble supervisors

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Professor of Oral and Maxillofacial Surgery
Faculty of Dentistry, Ain Shams University

& **Dr. Amr Amin**

Lecturer of Oral and Maxillofacial Surgery
Faculty of Dentistry, Ain Shams University

And to

**Dr. Ahmed Mohamed Abdellah**

Director of experimental surgery & animal research unit at medical research center, Ain Shams University

**Dr. Shaimaa Elsayed**

Assistant professor of Oral and Maxillofacial Radiology
Faculty of Dentistry, Ain Shams University

**Dr. Mona Hussien Raafat**

Assistant professor of Histology
Faculty of Medicine, Ain Shams University

**Dr.Yasser Elhadidi**

Assistant lecturer of Oral and Maxillofacial Surgery
Faculty of Dentistry, Ain Shams University

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| Abb. | Meaning |
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| **ASCs** | Adult stem cells |
| **BMP** | Bone morphogenic protein |
| **BMSCs** | Bone marrow stem cells |
| **ß-TCP** | ß-tricalcium phosphate |
| **BMC** | Bone marrow concentrate |
| **CFUFs** | Colony forming unit fibroblasts |
| **CADIA** | Computer Assisted Densitometric Image Analysis |
| **CCD** | Charge coupled device |
| **CM** | Conditioned media |
| **CT** | Computed tomography |
| **CBCT** | Cone beam computed tomography |
| **CTPs** | Connective tissue proginators |
| **DMEM** | Dulbecco's modified eagle's medium |
| **EMF** | Electromagnetic field |
| **ESCs** | Embryonic stem cells |

# List of Abbreviations

| Abb. | Meaning |
|---|---|
| **FBS** | Fetal bovine serum |
| **HA** | Hydroxyapatite |
| **H&E** | Haematoxylin and Eosin |
| **MSCs** | Mesenchymal stem cells |
| **µCT** | Micro computed tomography |
| **µm** | Micrometer |
| **NHA** | Nanocrystalline hydroxyapatite |
| **PI** | Pixel intensity |
| **PRP** | Platelet rich plasma |
| **PSP** | Photostimulable phosphor |
| **QCT** | Quantitative computed tomography |
| **RVG** | Radiovisiography |
| **ROI** | Region of interest |
| **2D** | Two dimension |
| **3D** | Three dimension |

# Abstract

**Objective:** The aim of the study was to compare the effect of the nanocrystalline hydroxyapatite and the stem cells in fracture healing.

**Materials and methods:** The study was conducted on twenty four skeletally mature New Zealand rabbits weighing 2 to 2.5 kg. The animals were divided equally into three groups. A 5 mm long osteotomy gap was created in the tibia of the animals after the fixation of the tibia with plate and screws. We added nanocrystalline hydroxyapatite (NHA) in the (NHA) group, injected mesenchymal stem cells (MSCs) into the osteotomy gap in the (MSCs) group and in the control group the osteotomy gap was left empty. All of the animals were sacrificed on postoperative day 22. Digital radiodensitometric analysis and histologic & histomorphometric examinations were performed on the harvested tibia. The data were statistically analyzed.

**Results:** Radiographic evaluation showed that there was no statistically significant difference found between NHA group and MSCs group, but there was highly statistically significant difference found between the NHA and the control group also between MSCs and control group. On the other hand, histologic and histomorphometric evaluation regarding trabecular bone thickness, we found that there was no statistically significant difference found between NHA group and control group, there was statistically significant difference found between NHA group and MSCs group and a highly significant difference between control group and MSCs group. Regarding area percentage of osteoid tissue, there was a highly statistically significant difference found between the three studied groups.Furthermore, there was a highly statistically significant positive correlation between trabecular bone thickness and bone density. On the other side, there was a highly statistically significant negative correlation between them and area percentage of osteoid tissue.

**Conclusion:** Mesenchymal stem cells showed promising results in improving bone quality in fracture healing on macroscopic & microscopic levels while nanocrystalline bone graft enhanced bone healing and this was radiographically very clear, but minimally microscopically.

**Keywords:** Bone, fracture, nanocrystalline hydroxyapatite, mesenchymal stem cells, MSCs.

# INTRODUCTION

Bone is a dynamic, vascularized tissue with a unique capacity to heal and remodel without leaving a scar[1]. Bone remodeling is the result of a balance between the activities of two different cell populations, the osteoclasts and the osteoblasts, which are responsible for bone resorption and deposition, respectively[2]. However, in certain situations including non-union fractures and diseases such as osteoporosis, osteoarthritis, cancer and infection the normal repair and remodeling processes are often impaired[3].

The question of how we can propaply manage extensive bone injuries is considered one of the dilemmas in oral and maxillofacial surgery. The traditional method that used to repair segmental bone defects is autogenous bone graft which is still considered the gold standard for bone transplantation. Autogenous bone graft possesses all the three elements for new bone growth: osteogenicity, osteoinductivity and osteoconductivity. Unfortunately, this method has many restrictions as limited supply, donor-site pain, infection, and bleeding, so novel approaches are being investigated[4–7].

It has been demonstrated that nanostructured materials may promote greater amounts of specific protein interactions and more efficiently stimulating new bone formation compared with conventional materials. It has also been indicated that, when features or ingredients of scaffolds are nanoscaled, a variety of interactions can be stimulated at the cellular level[8]. Recently, a new synthetic nanocrystalline hydroxyapatite graft material has been introduced for augmentation procedures in osseous defects.

The advantages of nanostructured material in comparison to bulk material are the close contact with surrounding tissues, quick resorption characteristics and a high number of molecules on the surface. In vivo studies demonstrated rapid healing of critical size defects after application of nanocrystalline hydroxyapatite (NHA) graft material[9].

Mesenchymal stem cells (MSCs) have inherent self-renewal and multipotent differentiation capacities. Bone marrow stem cells (BMSCs) possess outstanding promise for skeletal tissue regeneration therapies due to their easy & convenient isolation, immune-modulatory capability, ability to create a microenvironment that is favorable for tissue repair, less ethical concerns and lower risk of tumorigenesis as compared to embryonic stem cell [10,11].

Although there were many studies that have been conducted to evaluate the effect of nanocrystalline hydroxyapatite and mesenchymal stem cells in bone healing, there is lack of research comparing the effect of NHA and MSCs in fracture healing.

# REVIEW OF LITERATURE

Bones are organs of the skeletal system that give shape, mechanical support & protection to the body, facilitating the movement, participate in the mineral homeostasis and endocrine regulation[12]. Furthermore, they are continuously being remodeled throughout life[13].

Normal bone remodeling, is necessary for fracture healing and skeleton adaptation to mechanical use, as well as for calcium homeostasis, which occurs through the concerted actions of bone cells (osteoblasts, osteoclasts and osteocytes). Osteoblasts are responsible for bone formation. On the other hand, bone resorption is performed by osteoclasts, whereas osteocytes act as mechanosensors and orchestrators of the bone remodeling process. The remodeling process is under the control of local and systemic factors that all together contribute in bone homeostasis[14].

Osteoblasts are mature metabolically active bone forming cells. They secrete osteoid, the unmineralized organic matrix that subsequently undergoes mineralization, giving the bone its strength and rigidity. As their bone forming activity nears completion, some osteoblasts are converted into osteocytes, whereas others remain on the periosteal or endosteal surfaces of bone as lining cells. Osteoblasts also play a role in the activation of bone resorption by osteoclasts[15].

Osteocytes are mature osteoblasts trapped within the bone matrix. Even though the metabolic activity of the osteoblast decreases once it is fully encased in bone matrix, they still produce matrix proteins. Osteocytes have numerous long cell processes rich in microfilaments that are organized during

the formation of the matrix and before its calcification. They form a network of thin canaliculi permeating the entire bone matrix. The exact function of these cells remains obscure. It is likely that osteocytes respond to bone tissue strain and enhance bone remodeling activity by recruiting osteoclasts to sites where bone remodeling is required[13].

Osteoclasts are the only cells that are known to be capable of resorbing bone. Activated multinucleated osteoclasts are derived from mononuclear precursor cells of the monocyte-macrophage lineage. It is usually found in contact with a calcified bone surface and within a lacuna (Howship's lacunae) as a result of its own resorptive activity[16].

A bone fracture causes a break in the bone, which results in losing anatomic continuity and/or mechanical instability of the bone. It is a common sequela that is happened as a result of falls, car accidents or sports injuries. Moreover, it is related to penetration injuries or metabolic diseases of bone such as osteoporosis[17].

Fracture healing is a natural process that can reconstitute injured tissue and recover its original function and form. It is a very complex process that involves the coordinated participation of immigration, differentiation and proliferation of inflammatory cells, angioblasts, fibroblasts, chondroblasts and osteoblasts. These cells synthesize and release bioactive substances which contribute to the extracellular matrix components (e.g., different types of collagen and growth factors). Two categories of fracture healing exist (primary or secondary fracture healing). Primary healing occurs in cases of extreme stability and negligible gap size, involving a direct attempt by the bone to form itself