

A Comparative Study on the Efficacy of Acellular Dermal Graft versus Propylene Mesh Both Loaded with BM-MSCs in Healing of Skull Bone Defect in Adult Albino Rats

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BY

Lobna Abd El Razik M. Elkhateb

Assistant lecturer of Histology and Cell Biology
M.B.B.ch & M.Sc. Histology and Cell Biology

Under Supervision of

Professor Dr. / Adel Salah El-din Zohdy

Professor of Histology and Cell Biology

Faculty of Medicine, Ain Shams University

Professor Dr. / Suzi Sobhy Atalla

Professor of Histology and Cell Biology

Faculty of Medicine,, Ain Shams University

Professor Dr. / Manal Hassan Moussa

Professor of Histology and Cell Biology

Faculty of Medicine, Ain Shams University

Assistant professor Dr. / Ghada Galal Hamam

Assistant professor of Histology and Cell Biology

Faculty of Medicine, Ain Shams University

Dr. / Fatma Abd Elkarim Abu Zahra

Fellow in molecular biology of Tissue Culture

**Faculty of Medicine
Ain Shams University**

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**دراسة مقارنة بين كفاءة الرقعة الادمية اللاخلوية وشبكة
البروبيلين المحملتين بالخلايا الجذعية المستنبطة من نخاع العظم
في التئام خلل عظم الجمجمة في الجرذان البيضاء البالغة**

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مقدمة من

الطبيبة/ لبنى عبد الرازق محمد الخطيب
مدرس مساعد بقسم الهستولوجيا وبيولوجيا الخلية

تحت إشراف

أ.د/ عادل صلاح الدين زهدي
أستاذ بقسم الهستولوجيا وبيولوجيا الخلية
كلية الطب - جامعة عين شمس

أ.د / سوزي صبحي عطاالله
أستاذ بقسم الهستولوجيا وبيولوجيا الخلية
كلية الطب - جامعة عين شمس

أ.د / منال حسن موسى
أستاذ بقسم الهستولوجيا وبيولوجيا الخلية
كلية الطب - جامعة عين شمس

أ.م. د / غادة جلال حمام
أستاذ مساعد بقسم الهستولوجيا وبيولوجيا الخلية
كلية الطب - جامعة عين شمس

الدكتورة/ فاطمة عبد الكريم أبو زهرة
زميل في البيولوجيا الجزيئية لزراعة الانسجة

كلية الطب
جامعة عين شمس

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List of abbreviation

ADM	Acellular Dermal Matrix
BM-MSCs	Bone Marrow Derived Mesenchymal Stem Cells
BMPs	Bone Morphogenetic Proteins
DAB	Diaminobenzidine tetrahydrochloride
DMEM	Dulbecco's Modified Eagles Medium
EDTA	Ethylene Diamine Tetraacetic Acid
FBS	Fetal Bovine Serum
HRP	Horse-radish peroxidase
NaCl	Sodium Chloride
PBS	Phosphate Buffer Saline
SEM	Scanning Electron Microscope
SDS	Sodium Dodecyl Sulfate
SD	Standard Deviation
TNF-α	Tumor Necrotic Factor α

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Abstract

Introduction: Bone defects occur after tumor resection, post trauma, or in developmental deformities. Diseased bones do not heal properly and this leads to reduced quality of life with social and psychological problems. Bone-tissue engineering had emerged as an alternative method to autologous or allogeneic bone grafts.

Aim: to evaluate the efficacy of ADM versus propylene mesh both loaded with BM-MSCs in healing of skull bone defect in adult albino rats.

Materials and methods: the study included 40 male albino rats. Five weaned male albino rats were used for preparation of BM-MSCs. Five adult rats were used to obtain the dermal graft. Thirty adult rats weighing 150- 200 gm were divided into three groups according to the way of filling skull bone defect. **Group I: Ia** (-ve control), **Ib** (spontaneous healing). **Group II: IIa** (unseeded propylene), **IIb** (seeded propylene). **Group III: IIIa** (unseeded ADM), **IIIb** (seeded ADM).. The trephine operation was done on the left parietal bone. Specimens were collected four weeks postoperative and processed for H&E, osteopontin immunohistochemistry and scanning electron microscope. Morphometric and statistical analysis were also performed.

Results: bone defects filled with seeded scaffolds showed significant progression in new bone formation than unseeded scaffolds. Seeded grafts showed a significant

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increase in the mean percentage osteoblastic surface, mean thickness of osteogenic layer at the edge of the defect and area percentage of osteopontin expression. Seeded ADM showed significant increase in histomorphometric parameters compared to seeded propylene mesh.

Conclusions: It was concluded that seeded ADM provides strong support for new bone formation in clavicular bone defect.

Introduction

Introduction:

Although bone is one of the tissues that has an intrinsic ability to regenerate, yet isn't able to repair itself in large defects. In addition, in cases of severe trauma, developmental deformities, and tumor resection, diseased bones do not heal properly. This leads to reduced quality of life in many patients (**Ana et al., 2011**).

In these cases, autogenous bone graft (bone from the patient) has been the implant of choice. However, autogenous and allogenic bone grafts (bone from another human) have several limitations, such as donor-site infection, pain, and disease transfer (**Öztürk, et al., 2006**). Moreover, it's an expensive procedure, there is a limited supply of tissue and it causes significant donor-site morbidity (**Ana et al., 2011**). Xenografts (bone from other species) are also used but are not ideal substitute as they may lead to immunogenicity, disease transmission, rejection, infection and toxicity (**Javaid and Kaartinen, 2013**).

To overcome these problems, tissue engineering has emerged as a new approach for bone reconstruction. Synthetic materials or natural polymers are used as scaffolds to fill the bony defect. They have the advantage of being osteoconductive media and they also abolish the risk of infection or rejection (**Zohreh et al., 2012**).

Propylene mesh is one of the synthetic materials that can be used as scaffolds to substitute lost tissues as it has many

Introduction

advantages. It is made of an inert, single-stranded, flexible material that can be easily adapted to the receptor area. It does not suffer any fragmentation, is highly resistant to traction and can be sterilized by autoclaving. It represents low toxicity to the tissues, does not maintain an acute inflammatory process so can be used in infected areas (*Elvidio et al., 2001*).

On the other hand, acellular dermal graft (ADM) is considered as natural material that can replace soft tissues. It is prepared by decellularization of skin through a special process that preserves the bioactive components without damaging the extracellular dermal matrix and basement membrane architecture. The resulting graft serves as a framework to support cellular repopulation, revascularization at the surgical site, and lost tissue regeneration by the recipient's own cells (*Zohreh et al., 2012*).

To provide osteoinductivity (the ability to differentiate osteogenic into osteoblasts), scaffold materials can be loaded with osteogenic cells in order to generate a living bone graft *in vitro*. Osteoblasts obtained from autologous bone biopsies and then expanded *in vitro* were an obvious first choice due to their non-immunogenicity. However, their relatively low number, the time-consuming and the problems associated with obtaining osteoblasts from patients with bone-related diseases, are all disadvantages to this procedure. Mesenchymal stem cells (MSCs) which can be obtained from bone marrow (BM) provide an alternative source of osteogenic cells. They can be extensively

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expanded *in vitro* in large numbers sufficient to treat large bone defects. They have immunosuppressive effects *in vivo*, which may make them suitable for allogeneic transplantations (**Anindita et al., 2010**).

When this approach of bone reconstruction used to repair or reshape the face and skull of a living person; it is termed as **craniofacial reconstruction**. In children, craniofacial reconstruction is done to repair abnormalities in the shape of the child's skull and facial features resulting from birth defects or genetic disorders (**Mark and Robert, 1999**). While in adults, it is done following head or facial trauma, or in cancer patients who have lost part of the bony structures of the face following tumor surgery (**Stephen, 2000**).

This procedure is complicated as the surgeon is operating on a part of the body containing important structures as; brain, upper part of the spinal cord, eyes and opening of the airway within a small space (**Leong et al., 2002**).

Aim of this work:

To evaluate the efficacy of ADM versus propylene mesh both loaded with BM-MSCs in healing of large skull bone defect in adult albino rats.

Review of literature

I-Bone Histology

Bone types:

Bone is the basic unit of the human skeletal system that provides the framework for the weight of the body, protects the vital organs, supports mechanical movement and hosts stem cells including hematopoietic cells. According to histological structure, bone can be classified into compact bone and cancellous bone. Compact bone is ivory like and dense in texture without cavities. It consists mainly of haversian systems or osteons. It is the shell of many bones and surrounds the cancellous bone in the center. Meanwhile cancellous bone is sponge like with numerous cavities. It is located within the medullary cavity and consists of extensively connected bony trabeculae that are oriented along the lines of stress. Cancellous bone has larger surface area than compact bone, so it is more metabolically active for remodeling. Cancellous bone receives its blood supply from two freely anastomosing systems; one comes from the periosteum, the other from large nutrient arteries that penetrate directly into the medullary bone (*Adler, 2000*).

Cancellous bone showed anisotropy; it appears as irregularly organized rods and plates. While cortical bone appears as highly compact and orthotropic (regularly arranged) due to the circular nature of the osteons that make up its structure. On the micro-architectural level, cortical bone contains only microscopic channels through the center of the osteons whereas cancellous bone is highly porous. Mechanically, cortical bone provides support for

Review of literature

the overall bone structure, while cancellous bone acts as a shock absorber and resists compressive loads. The mechanical strength depends on several geometric parameters as trabecular thickness, density, and bone surface to bone volume ratio (*Liebschner and Wettergreen, 2003*).

Bone is a highly dynamic and complex tissue exposed to various stimuli throughout lifetime. It plays crucial roles in both mechanical support and mineral homeostasis. Within a skeletal element, compact bone represents 80% of the mass of an adult human skeleton. It shows only 10% porosity. While spongy bone appears with 50%–90% porosity, these spaces are filled with BM (*Costa-Pinto, et al., 2011*).

Bone structure:

Bone is a complex and highly organized mineralized tissue. It is formed of bone matrix which contains different type of cells. Mostly, all bones are covered by the periosteum which is a highly vascularized fibrous connective tissue. Maintenance and remodeling of bone is carried out by the action of five different cell types which are bone-lining cells, osteogenic cells, osteoblasts, osteocytes and osteoclasts. Within the bone structure, MSCs are found in the BM and also in the periosteum (*Jee, 2001*).

Bone matrix consists of organic and inorganic components which in combination gives bone its hardness and resistance. The organic component is composed of collagen fibers with predominately type I collagen (95%) arranged in alternate direction according to the pressure

Review of literature

applied to bone. It also contains amorphous material, including glycosaminoglycans that are associated with non collagenous proteins. This uncalcified organic matrix is known as osteoid. Inorganic matter represents about 50% of the dry weight of bone matrix. It is composed of hydroxyapatite crystals (calcium and phosphorus), as well as small amounts of bicarbonate, citrate, magnesium, potassium, and sodium (*Balcerzak, et al., 2006*).

Non collagenous protein content found in bone is known as **SIBLING** (small integrin-binding ligand, *N*-linked glycoprotein). They have role in bone formation, growth and repair, cellular adhesion to the matrix, mineral nucleation and mineral maturation. They include bone sialoprotein, **osteopontin (OPN)**, and the calcium binding subdomain of dentin sialophosphoprotein, dentin phosphoprotein. Osteopontin is a highly phosphorylated sialoprotein and has high calcium binding potential. It is produced by osteoblasts at various stages of differentiation, osteocytes and osteoclasts. Osteopontin was observed to be normally expressed in bone matrix around blood vessels, in cement lines, and at the endocranial (dura surface) and ectocranial (periosteal surface), as well as in the cytoplasm of osteoblasts and osteocytes (*George and Veis 2008*).

Osteoblasts are cuboidal and flat cells and are nearly 20 μm in diameter. They are differentiated from MSCs present in BM. They are metabolically active mature bone-forming cells. Osteoblasts produce a disorganized (or woven) osteoid matrix of type-1 collagen, which undergoes