شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

# بسم الله الرحمن الرحيم


HANAA ALY

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# جامعة عين شمس

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# قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

# يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

HANAA ALY


# Decellularized Periosteum of New Zealand Adult Rabbits as a Potential Biologic Scaffold for Bone Tissue Engineering:

## Histological and Immunohistochemical Study

*Thesis*

*Submitted for Partial Fulfillment of M.Sc. Degree in Histology and Cell Biology*

*By*

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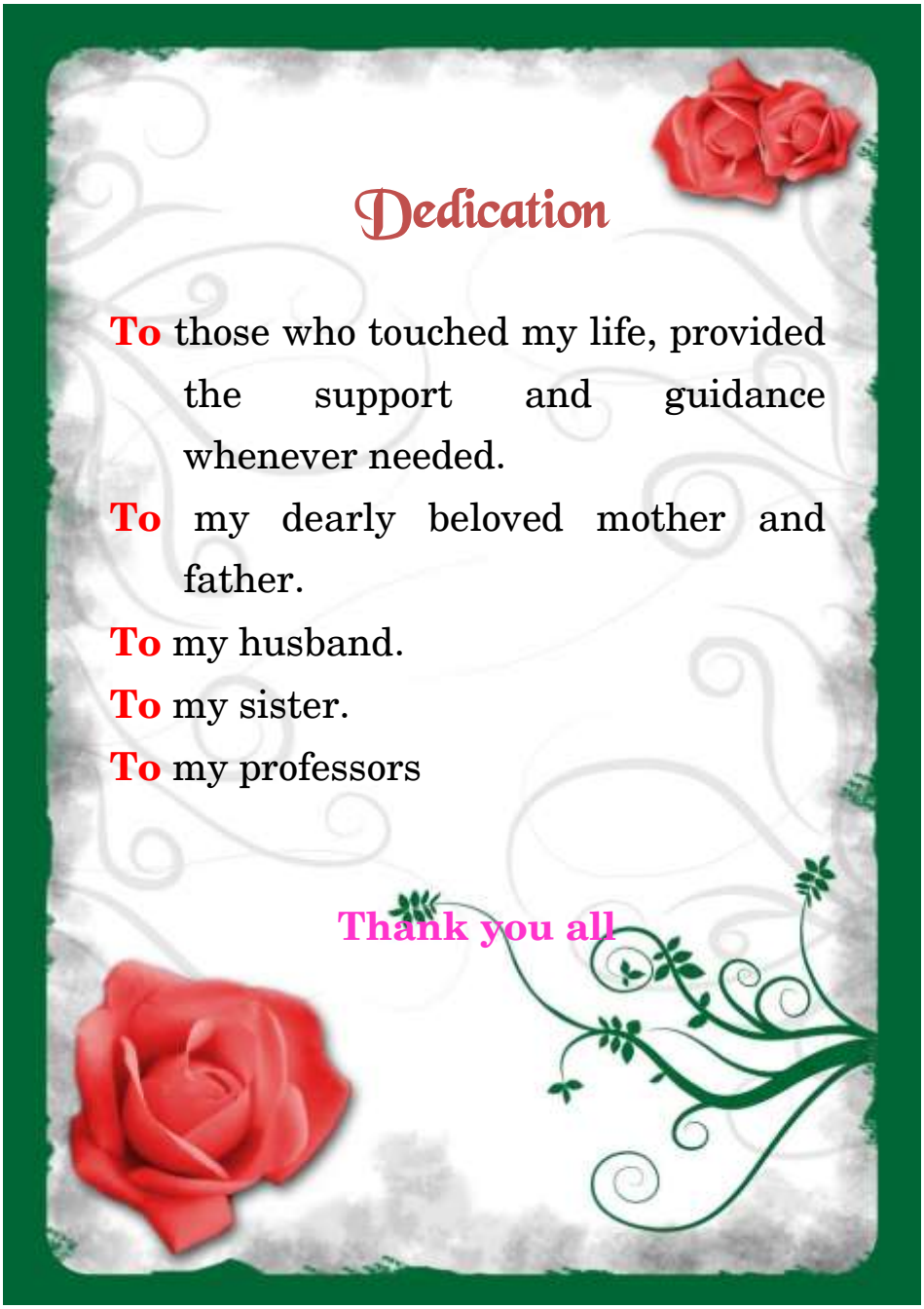
*Manal Ashour*



# Dedication

**To** those who touched my life, provided the support and guidance whenever needed.

**To** my dearly beloved mother and father.

**To** my husband.

**To** my sister.

**To** my professors

**Thank you all**

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| **3-D** | Three-dimensional |
| **ADM** | Adriamycin |
| **AETI** | Alveolar epithelial type I |
| **AETII** | Alveolar epithelial type II |
| **bFGF** | Basic fibroblast growth factor |
| **BM-MSCs** | Bone marrow–derived mesenchymal stem cells |
| **BMP** | Bone morphogenetic protein |
| **CD** | Cluster of differentiation |
| **chitosan-DP globules** | Decellularized periosteum with chitosan globules |
| **CM** | Conditioned medium |
| **COX-2** | Cyclooxygenase type II |
| **DAB** | Diaminobenzidine |
| **DAPI** | 4, 6-Diamidino-2-phenylindole |
| **dECM** | Decellularized ECM |
| **DMEM F12** | Dulbecco's Modified Eagles Medium F12 |
| **D-periosteum** | Decellularized periosteum |
| **ECM** | The extracellular matrix |
| **EDTA** | Ethylene Diamine Tetra Acetic Acid |
| **FasL** | Fas ligand |
| **FBS** | Foetal bovine serum |
| **Fzd** | Frizzled |
| **GAG** | Glycosaminoglycans |

| Abb. | Full term |
|---|---|
| **GDF-5** | Growth differentiation factor |
| **GFs** | Growth factors |
| **hESCs** | Human embryonic stem cells |
| **HPDCs** | Human periosteum derived cells |
| **IL** | Interleukin |
| **IL- 10** | Interleukin-10 |
| **iMSCs** | iPSC-derived mesenchymal stem |
| **iPSCs** | Induced Pluripotent stem cells |
| **JPCs** | Jaw periosteal cells |
| **mES** | Murine embryonic stem cells |
| **mPDCs** | Murine PDCs |
| **MSCs** | Mesenchymal stem cells |
| **N-Periosteum** | Native periosteum |
| **PBS** | Phosphate Buffer Saline |
| **PC-Exos** | Exosomes derived from periosteum derived cells |
| **PCs** | Periosteum derived cells |
| **PDCs** | Periosteum derived cells |
| **PDGF** | Platelet-derived growth factor |
| **PDPCs** | Periosteum derived progenitor cells |
| **PGL** | Polyglycolide-co-polylactide |
| **Prx-1** | Paired-related homeobox gene-1 |
| **PTH** | Parathyroid hormone |
| **PTHR1** | Parathyroid hormone type I receptor |
| **PTHrP** | Parathyroid hormone-related protein |

| Abb. | Full term |
|---|---|
| **RPM** | Round per minute |
| **RUNX2** | Runt-related transcription factor 2 |
| **sdf-1** | Stromal cell-derived factor-1 |
| **SDS** | SODIUM dodecyl sulfate |
| **SIS** | Small intestinal submucosa |
| **SMA9** | Smooth muscle actin 9-expressing cells |
| **Sox9** | Sry-related high-mobility group box 9 |
| **TBS** | Tris-buffered saline |
| **TEP** | Tissue-engineered periosteum |
| **TGF** | Transforming growth factor |
| **TGFBR2** | Transforming growth factor Beta receptor 2 |
| **TGFBR1** | Transforming growth factor-beta type I receptor |
| **TGF-β** | Transforming growth factor β |
| **TNF-α** | Tumor necrosis factor-alpha |
| **VEGF** | Vascular endothelial growth factor |
| **αSMA** | Alpha smooth muscle actin |
| **β-TCP** | β-tricalcium phosphate |

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# Abstract

**Background and aim of the study:**
Bone grafting is typically used to bridge a bone defect. Bone graft healing and remodeling is always a main interest of orthopedic surgeons. Because the periosteum has a significant regenerative capacity and is widely known to be essential for the initiation of bone graft healing and remodeling, this study was conducted to produce a rabbit decellularized periosteum to be used as a biologic scaffold for future bone tissue engineering. Periosteum-derived progenitor cells (PDPCs) could adhere, proliferate and infiltrate into the D-periosteum when combined together in *vitro*.

**Methods:** Twenty-five adult male New Zealand rabbits were divided into 4 groups. Group I (the native periosteum), Group II (the decellularized periosteum), Group III (PDPCs isolation, culture, and characterization), Group IV (the recellularization of the D-periosteum by PDPCs). Native and decellularized periosteum were prepared for histological and immunohistochemical techniques. Samples of recellularized periosteum were taken at days 3, 7, 10 after cell seeding and sections were stained with H&E and toluidine.

**Results:** Light microscopic examination revealed absence of cell nuclei in the D-periosteum as compared with the N-periosteum and this was demonstrated by using H & E staining, DAPI staining and agarose gel electrophoresis. The distribution of collagen fibers in periosteal layers were preserved after decellularization. However, the glycosaminoglycans in periosteal layers decreased. Light microscopic examination after recellularization revealed that PDPCs could adhere, proliferate and infiltrate into the D-periosteum in *vitro*. Moreover, osteoid tissue was observed, and this was demonstrated by toluidine blue staining.

**Conclusion:** The D-periosteum maintains biocompatibility in *vitro*, therefore, can provide a naturally compatible scaffold for bone tissue engineering in future.

**Keywords:** Decellularization, Periosteum, Extracellular matrix, Bone tissue engineering, Periosteum-derived progenitor cells (PDPCs).