



Ain Shams University  
Women's College for Arts,  
Science and Education  
Zoology Department

# **Safety Evaluation of Some Polymers, Reinforced by Bioglass to Enhance Bone Regeneration in Albino Rats.**

PhD Thesis  
**Doctor of Philosophy in Science**  
(Zoology)

By

**Biologist/ Karam Ibrahim Radwan Eldesoqi**

Research Assistant of Biomaterials, Biomaterials Dept., National  
Research Center, Cairo, Egypt.

PhD Student in Zoology Dept., Women's College for Arts, Science  
and Education, Ain Shams University, Cairo, Egypt.

Guest PhD Student in Trauma, Hand & Reconstructive Surgery Dept.,  
Faculty of Medicine, Johann- Wolfgang- Goethe University,  
Frankfurt/ Main, Germany.

2013



Ain Shams University  
Women's College for Arts,  
Science and Education  
Zoology Department

### **Board of Scientific Supervision in Ain Shams University, Cairo, Egypt.**

Prof. Dr. **Karima Mohammad Sweify** :- Professor of Cytogenetic, Zoology Dep.t, Women's College for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

Prof. Dr. **Bothaina Mohammad Abd El Hady**: - Professor of Polymers & Biomaterials, Biomaterials Dept., National Research Center, Cairo, Egypt.

Prof. Dr. **Mahmoud Sayed Soliman Arbid**:- Professor of Pharmacology and Toxicology, Pharmacology Dept., National Research Center, Cairo, Egypt.

Dr. **Abeer Mahmoud Al-Kady**:- Researcher of Biomaterials, Biomaterials Dept., National Research Center, Cairo, Egypt.

### **Board of Scientific Supervision during DAAD Scholarship in Johann-Wolfgang-Goethe University, Frankfurt/Main, Germany.**

Prof. Dr. med. **Ingo Marzi**:-Professor and Head of Trauma, Hand & Reconstructive Surgery Dept., Faculty of Medicine, Johann-Wolfgang-Goethe University, Frankfurt/Main, Germany.

Dr. med. habil. **Caroline Seebach**:- Assistant Professor of Orthopaedics and Traumatology in Trauma, Hand & Reconstructive Surgery Dept., Faculty of Medicine, Johann-Wolfgang-Goethe University, Frankfurt/ Main, Germany.

Dr. phil.nat. habil. **Dirk Henrich**:- Assistant Professor and Head of Scientific Laboratories in Trauma, Hand & Reconstructive Surgery Dept., Faculty of Medicine, Johann- Wolfgang- Goethe University, Frankfurt/Main, Germany.



Ain Shams University  
Women's College for Arts,  
Science and Education  
Zoology Department

## Approval Sheet

**Name:** Karam Ibrahim Radwan Eldesoqi

**Title :** Safety Evaluation of Some Polymers, Reinforced by Bioglass to Enhance Bone Regeneration in Albino Rats.

### Board of Scientific Supervision in Ain Shams University.Cairo, Egypt.

Prof. Dr. **Karima Mohammad Sweify** :- Prof. of Cytogenetic, Zoology Dept., Women's College for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

Prof. Dr. **Bothaina Mohammad Abd El Hady** :- Prof. of Polymers & Biomaterials, Biomaterials Dept., National Research Center, Cairo, Egypt.

Prof. Dr. **Mahmoud Sayed Soliman Arbid**:- Prof. of Pharmacology and Toxicology Pharmacology Dept., National Research Center, Cairo, Egypt.

Dr. **Abeer Mahmoud Al-Kady**:- Researcher of Biomaterials, Biomaterials Dept., National Research Center, Cairo, Egypt.

**Head of Zoology Department**

## Acknowledgement

First and foremost thanks are due to GOD the most beneficial and merciful.

My sincere appreciation goes to my supervisor, Professor **Ingo Marzi**, for wise supervision and unconditional support till PhD project and thesis were accomplished in Germany, also his attitude to research inspired me to continue to a PhD program and want to be a member of the academic family.

I would like to express my deep gratitude and respect to Dr. **Caroline Seebach** whose supervision, advices and insight was valuable to me. Additionally, I would like to thank Dr. **Dirk Henrich** whose I learned from him, and for providing the Vision Lab for the experiments, and his ability to put complex ideas into simple terms.

Furthermore, many doctors and colleagues at the laboratory and the Department of Traumatology have contributed to an outstanding and diligent scientific team, which in countless ways assisted me in my thesis.

I would like to express my deepest gratitude to my supervisor Professor **Karima Sweify**, for her instructive guidance, valuable assistance and constructive criticism. I am so grateful for her support, assistance, reading and criticizing the manuscript. It is a great honor to work under her supervision.

My sincere appreciation goes to my supervisor Professor **Bothaina Abd El Hady** for her unconditional support to gain and extensions of my DAAD scholarship, also her continuous help, thesis revision and support in all stages of my thesis in Egypt, as well as in Germany.

I would also like to thank my supervisor Professor **Mahmoud Soliman Arbid** for being an open person to ideas, and for encouraging and helping me to shape my interest and ideas in my PhD project, I greatly benefited from his keen scientific insight, his knack for solving seemingly intractable practical difficulties



My sincere appreciation goes to my supervisor Dr. **Abeer Al-Kady** for her help and support to start my PhD thesis in Egypt, also for encouraging and helping me to apply for the DAAD scholarship in order to complete my PhD thesis in Germany.

Most of all, I thank all my colleagues who supported and helped me to accomplish my PhD thesis at the Frankfurt University, DAAD, Ain Shams University and National Research Center. I would also like to thank all the people who contributed in some way to the work described in this thesis.

At the end, I would like to thank my family, especially my mother, father and my wife for their continuous love, encouragement and support to achieve my hopes and dreams.

**Karam Eldesoqi**

2013, Frankfurt, Germany



Ain Shams University  
Women's College for Arts,  
Science and Education  
Zoology Department

## Qualification

Name	Karam Ibrahim Radwan Eldesoqi
Scientific Degree	M.Sc. In Zoology
Department	Zoology
College	Women's College for Arts, Science and Education
University	Ain Shams University



Ain Shams University  
Women's College for Arts,  
Science and Education  
Zoology Department

## Dedication

I dedicate this work to GOD, my dear mother,  
father and wife who encouraged me during this work;  
I will not forget their continuous support.

## **Abstract**

Bone grafts are performed to enhance healing of spine fusions and fractures and to regenerate bone in osseous defects and malformations. Bone tissue engineering provides a promising therapeutic option to improve the local bone healing response.

In the present study the possibility of using composite materials consisting of polymer [poly (L-lactide) (PLLA)] and bioglass (particles sizes 125 – 106  $\mu\text{m}$ ) as scaffold was investigated, Also loaded scaffolds with undifferentiated or differentiated Mesenchymal stem cells (MSCs) and Endothelial progenitor cells (EPCs) in bone tissue engineering.

Bioglass and PLLA were mixed for the preparation of bioglass scaffolds: PLA, BG 20% and BG 40%; (0, 20 and 40 %wt bioglass respectively). The scaffolds were in the form of disc shape 5 mm diameter and 1 mm thickness.

80 Adult male albino (Sprague Dawely Strain) rats weighing approximately 350-450g, were used and distributed in 11 groups; Control group; Negative control (defect) group; 3 groups were implanted only with PLA , BG 20 and BG 40 scaffolds; another three groups were implanted with PLA , BG 20 and BG 40 Scaffolds loaded with mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs); The last 3 groups were implanted with PLA , BG 20 and BG 40 scaffolds were loaded with differentiated mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs).

Osteoconductive properties of implanted specimens were evaluated by using the Skull Critical Size defect (CSD) model, 6 mm diameter, for 14 weeks. After sacrifice cranium were removed for Micro CT analysis, histological (H&E) and histomorphometrical evaluation.

The area of new bone formation increased by increasing the percent of bioglass in the scaffold. This might be due to the release of calcium and silica ions which played an important role in the bone healing process.

EPCs and MSCs showed synergetic effect using bioglass scaffold by two mechanisms: EPCs increased micro vessel infiltration





which lead to increase of blood supply and oxygen to forming new bone tissue, also MSCs were differentiated and proliferated to osteocytes, which accelerate new bone tissue formation, especially when MSCs were predifferentiated to differentiated MSCs before loading the scaffold.

No behavioral changes or visible signs of physical impairment or neurological toxicity were observed during the 14 weeks observation period. The macroscopic analysis of the implant sites demonstrated a comparable scar formation and subsequent healing process in all groups. Serum NO<sub>2</sub>, Leukocyte count, body temperature were determined with no systemic inflammation.

Liver (ALT, AST& ALP) and kidney (Urea & Creatinine) functions analysis showed no toxic effect. Occurrence of tumors was assessed macroscopically and histologically in slides of liver, kidney and spleen. Furthermore, the concentrations of malondialdehyde (MDA), sodium oxide dismutase (SOD) and total glutathione (GSH) concentration in the serum were used as indicators of free radicals activity. Previous analysis showed no organ damage, no systemic inflammatory reactions were assessed and no free radicals liberation.

In conclusion, Bioglass (particles sizes 125–106  $\mu\text{m}$ ), as scaffold, supports the bone formation and further enhanced in presence of EPC and MSC and more with d.MSCs. Bioglass scaffolds showed a good biocompatibility in *vitro* and in *vivo*, also didn't liberate free radicals. Furthermore additional EPC and MSC seeded onto the scaffold did not show side effects in *vivo*.

## Contents

Chapter	Page
Tables List.....	- I
Figures List.....	- II
Abbreviations List.....	- V
<b>I- Introduction.....</b>	<b>- 1</b>
Aim of the work.....	- 3
<b>II- Review of the literature.....</b>	<b>- 4</b>
<b>1. Tissue Engineering Scope.</b>	<b>- 4</b>
<b>2. Bioglass Scaffold.</b>	<b>- 7</b>
2.1. Mechanisms of bioactivity.	- 7
2.2. Polymers, (PLA).	- 9
2.3. Composites.	- 10
2.4. Ionic dissolution products, Osteogenesis and Angiogenesis.	- 11
<b>3. Endothelial Progenitor Cells (EPCs).</b>	<b>- 12</b>
3.1. EPC Identification.	- 12
3.2. EPC Characterization.	- 13
3.3. EPC Types.	- 13
3.4. EPC function.	- 14
3.5. EPC Regulation.	- 14
3.6. EPC Mechanism.	- 15
3.7. The application of EPC-based therapies.	- 15
<b>4. Mesenchymal Stem Cells (MSC).</b>	<b>- 16</b>
4.1. Marrow Stromal cells.	- 17
4.2. Identification and Characterization of MSCs.	- 18
4.3. Isolation of MSC.	- 19
4.4. Osteogenic Differentiation of MSC.	- 21
4.4.1. Dexamethasone (Dex), $\beta$ -glycero-phosphate ( $\beta$ -GP).	- 22



4.4.2.	Bone morphogenetic proteins (BMPs).	- 22
4.4.3.	Scaffold or Matrix.	- 23
4.5.	Migration and Homing.	- 23
4.6.	Factors influencing MSC.	- 24
4.6.1.	Age.	- 24
4.6.2.	Gender .	- 24
4.6.3.	Mediators.	- 24
4.7.	Mechanism of Action.	- 25
4.8.	MSC Application in Bone Diseases.	- 27
<b>5.</b>	<b>A critical size defect (CSD) model</b>	<b>-28</b>
<b>6.</b>	<b>Toxicology</b>	<b>-29</b>
<b>III</b>	<b>Material and methods.....</b>	<b>- 33</b>
<b>A.</b>	<b>Scaffold.</b>	<b>- 33</b>
<b>B.</b>	<b>Stem Cell Isolation, Preparation and Culture.</b>	<b>- 43</b>
1.	Isolation of human Endothelial Progenitor Cells (hEPCs) from Buffy Coat.	- 43
2.	Isolation of Rat Endothelial Progenitor Cells (rEPCs) from Rat Spleen.	- 43
3.	Isolation of Rat Mesenchymal Stem Cells (rMSCs) from Rat Femur.	- 35
4.	Differentiation of Rat Mesenchymal Stem Cells (rMSCs).	- 35
<b>C.</b>	<b>Scaffold Biocompatibility Assessment and Toxicology in Vitro.</b>	<b>- 36</b>
1.	Seeding Efficacy Assessment of Bioglass Scaffolds seeded with Stem cells (rEPCs).	- 36
2.	Viability Assessment of Stem Cells (rEPCs or rEPCs + rMSCs) after seeding in Bioglass Scaffolds.	- 37
3.	Toxicology of Bioglass Scaffolds loaded with Stem Cells (hEPCs) in Vitro.	- 37
4.	Scanning Electron Microscopy (SEM) of rEPCs and rMSCs seeded on BG Scaffolds.	37



<b>D. Osteogenic Activity Evaluation of Bioglass Scaffolds loaded Stem Cells (rEPCs and rMSCs) in Vivo.</b>	<b>- 38</b>
1. Animals and Cell Transplantation.	- 38
2. Skull $\mu$ CT, Histology & Histomorphometry.	- 39
<b>E. Toxicology Evaluation of Bioglass Scaffolds loaded with Stem Cells (rEPCs and rMSCs) in Vivo.</b>	<b>- 40</b>
1. Blood Sampling and Internal Organ Collecting.	- 40
2. Liver, Kidney and Spleen Histology.	- 40
3. Liver and Kidney Function.	- 41
4. Inflammatory Activity & Free Radical Biomarkers Determination.	- 41
5. Haematological test.	- 44
<b>F. Statistics.</b>	<b>- 44</b>
<b>IV. Results.....</b>	<b>- 45</b>
<b>A. Scaffold.</b>	<b>- 45</b>
<b>B. Stem Cell Isolation, Preparation and Culture.</b>	<b>- 46</b>
<b>C. Scaffold Biocompatibility Assessment and Toxicology in Vitro.</b>	<b>- 49</b>
1. Seeding Efficacy Assessment of Bioglass Scaffolds seeded with Stem cells (rEPCs).	- 49
2. Viability Assessment of Stem Cells (rEPCs + rMSCs) after seeding in Bioglass Scaffolds.	- 51
3. Toxicology of Bioglass Scaffolds loaded with Stem Cells (hEPCs) in Vitro.	- 53
4. Scanning Electron Microscopy (SEM) of rEPCs and rMSCs seeded on BG scaffolds.	- 64
<b>D. In-vivo osteogenic activity evaluation of Bioglass scaffolds loaded stem cells (rEPCs and rMSCs).</b>	<b>- 68</b>
1. Animals and Cell Transplantation.	- 68
2. Skull $\mu$ CT, Histology & Histomorphometry.	- 70



<b>E. In-vivo toxicology evaluation of Bioglass scaffolds loaded with stem cells (rEPCs and rMSCs).</b>	<b>- 77</b>
1. Haematological Test.	- 77
2. Inflammatory Activity.	- 80
3. Liver, Kidney and Spleen Weight percentage.	- 84
4. Liver and Kidney Function.	- 88
5. Liver, Kidney and Spleen Histology.	- 96
6. Free Radical biomarkers determination.	103
<b>V. Discussion.....</b>	<b>107</b>
Role of Bioglass in Bone Healing.	107
Role of MSC in bone healing.	109
Role of differentiated MSC in bone healing.	111
Role of differentiated EPCs in bone healing.	112
Biocompatibility and Toxicology Evaluation of Bioglass Scaffolds loaded with Stem Cells (rEPCs and rMSCs).	114
Biocompatibility of PLA, BG20 and BG40.	114
Biodegradation.	117
Systemic Toxicology.	118
<b>VI English summary.....</b>	<b>120</b>
<b>VII References.....</b>	<b>125</b>
<b>VIII Arabic summary.</b>	

## Tables List

<b>Table</b>	<b>Legend</b>	<b>Page</b>
<b>1:</b>	Animal Group design.	- 38
<b>2:</b>	Cell Count of rEPCs stained with DIL stain and seeded with ChronOS, BG scaffolds & medium containing Ca <sup>++</sup> ions for 1-5 days Anova.	- 59
<b>3:</b>	Cell Length of rEPCs Anova, rEPCs stained with DIL stain and seeded with ChronOS, BG scaffolds & medium containing Ca <sup>++</sup> ions for 1-5 days.	- 61
<b>4:</b>	Ca <sup>++</sup> Concentration measurements in the medium of rEPCs which were seeded with ChronOS & BG scaffolds for 6 days Anova.	- 63



## Figures List

<b>Fig.</b>	<b>Legend</b>	<b>Page</b>
<b>1:</b>	Bone marrow (BM) mesenchymal stem cells (MSCs).	- 19
<b>2:</b>	Role of SOD in Cellular Antioxidant Defense Mechanism.	-31
<b>3:</b>	Chemistry of the Griess Reagents.	-42
<b>4:</b>	Scheme of the Malondialdehyde (MDA) Assay.	-42
<b>5:</b>	Scheme of the Superoxide Dismutase Assay.	-43
<b>6:</b>	GSH recycling.	-44
<b>7:</b>	PLA, BG 20% & BG 40% Scaffolds.	- 45
<b>8:</b>	Von Kossa Stain of MSCs and differentiated rMSCs.	- 47
<b>9:</b>	Von Kossa Stain stained area % of MSCs and differenti-ated rMSCs.	- 48
<b>10:</b>	Adherence % of BG scaffold + rEPCs.	- 50
<b>11:</b>	rEPCs + rMSCs or d.MSCs seeded on PLA, BG 20 % & BG 40% scaffolds & stained with DIL & DAPI stain.	- 52
<b>12:</b>	Transwell inserts with membrane size 8 $\mu\text{m}$ size and 24 well plate.	- 55
<b>13:</b>	rEPCs stained with DIL stain and seeded with ChronOS, BG scaffolds & Medium have $\text{Ca}^{++}$ ions for 1-5 days.	- 56
<b>14:</b>	Cell Count of rEPCs stained with DIL stain and seeded with ChronOS, BG scaffolds & medium containing $\text{Ca}^{++}$ ions for 1-5 days.	- 58