Freeze-Dried Platelet Concentrate Loaded Thermo-Sensitive Chitosan Hydrogel Scaffolds for Periodontal Tissue Regeneration: Preparation and Characterization (In Vitro Study)

A Thesis

Submitted to the Faculty of Dentistry, Cairo University, in Partial Fulfillment of the Requirements of the Degree of

Doctor of Philosophy

in

Dental Materials Science

By

Mohamed Mahmoud Ammar

BDS (2001) – Alexandria University, EGYPT MSc (2011) – Lehigh University, USA Lecturer Assistant, Biomaterials Department, Faculty of Oral and Dental Medicine Future University in Egypt

> Faculty of Dentistry Cairo University (2017)

Supervisors

Prof. Taheya Ahmed Moussa

Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Prof. Sayed Hussein Saniour

Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Ass. Prof. Gihan Hafez Waly

Assistant Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Referees

Prof. Inas Sami Abd-elhamid

Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Prof. Sabry Abd-elhamid Elkorashy

Professor, Inorganic Chemistry Department, Faculty of Science, Suez Canal University

Prof. Taheya Ahmed Moussa

Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Prof. Sayed Hussein Saniour

Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Ass. Prof. Gihan Hafez Waly

Assistant Professor, Biomaterials Department, Faculty of Dentistry, Cairo University

Acknowledgment

I would like to convey my deep and sincere appreciation to Dr. Taheya Moussa, Professor, Biomaterials Department, Faculty of Dentistry, Cairo University, for her great supervision, valuable guidance and unlimited assistance and support. It was a great honor to work under her guidance and supervision.

I was also honored to be supervised by Dr. Sayed Saniour, Professor, Biomaterials Department, Faculty of Dentistry, Cairo University. I would like to thank him for his kind help and patience, and his endless willingness to teach and share his highly valued knowledge.

I would like to express my utmost gratitude to Dr. Gihan Waly, Assistant Professor, Biomaterials Department, Faculty of Dentistry, Cairo university, for her close supervision, kind suggestion and sincere guidance throughout this study, and who devoted a great deal of her time and effort to supply me by all the help I need to complete this work.

I want to thank Dr. Nagwa Roshdy, Professor, Biochemistry Department, Faculty of Medicine, Cairo University, for her help and assistance in this work. I would like to thank Dr. Abdelfattah Abdelkhalek, Labs Chief Manger, Faculty of Pharmacy, Future University and all his assistants for their sincere help at all times with great kindness and respect.

I would also like to put across my gratitude to my colleague Dr. Sherine Ahmed Hashem, lecturer, Biomaterials Department, Faculty of Oral and Dental Medicine, Future University, who has been a huge help and my back throughout the work, without her help this work would not have been completed.

I also owe the Biomaterials Departments in both Faculty of Oral and Dental Medicine, Future University and Faculty of Dentistry, Cairo University a great deal. All the members of those departments were very helpful and understanding.

Finally, I want to thank Future University that provided me with all the facilities that helped me throughout my work.

Thank you.

Dedication

I would like to dedicate this work to the soul of my father who would have been in a great joy living this moment with me and to my lovely dear mother for her patience and support.

Table of Contents

Title	Page
List of tables	v
List of figures.	vii
List of abbreviations	xi
Chapter 1: Review of literature	1
1.1. Introduction	1
1.2. Tissue engineering and regenerative medicine	3
1.3. Tissue engineering of the periodontium	6
1.3.1. Growth factor based approach	8
1.3.2. Cell based approach	10
1.3.3. Growth factor and cell combination approach	11
1.4. Chitosan	12
1.4.1. Chitosan structure and preparation	13
1.4.2. Chitosan biocompatibility	16
1.4.3. Chitosan biodegradation	18
1.4.4. Chitosan role in wound healing	21
1.4.5. Chitosan as a tissue engineering scaffold	22
1.4.6. Chitosan hydrogels	27
1.4.6.1. Advantages of chitosan hydrogel	27
1.4.6.2. Mechanisms of chitosan hydrogel formation	29
1.4.6.2.1. Physical interactions	30
1.4.6.2.2. Chemical interactions	32
1.5. Platelet concentrate	33
1.5.1. Platelets composition	35

Title	Page
1.5.2. Platelets concentrate preparation	37
1.5.3. Platelets concentrate benefits and applications	38
1.5.4. Factors affecting platelets concentrate performance	39
1.6. Stem cells	41
1.6.1. Dental-derived stem cell populations	43
1.6.2. Periodontal ligament stem cells (PDLSCs)	44
1.7. Literature systematic review	48
1.7.1. Search strategy	48
1.7.2. Selection criteria	49
1.7.3. Findings	49
Chapter 2: Research question and aim of the study	51
2.1. PICO	51
2.2. Research question	52
2.3. Aim of the study	52
Chapter 3: Materials and methods	53
3.1. Thermo-sensitive chitosan hydrogel preparation and	33
characterization	56
3.1.1. Chitosan powder deacetylation degree measurement	56
3.1.2. Thermo-sensitive chitosan hydrogel preparation	57
3.1.3. Thermo-sensitive chitosan hydrogel characterization .	59 5 0
3.1.3.1. Rheology measurements	59
3.1.3.2. Biodegradation rate	61
3.2. Freeze-dried platelet concentrate preparation	63

Title	Page
3.2.1. Preparation of the platelet concentrate	63
3.2.2. Freeze-drying of the platelet concentrate	65
3.3. Platelet concentrate loading and characterization	65
3.3.1. Platelet concentrate loading	65
3.3.2. Platelet concentrate loaded hydrogel characterization.	66
3.3.2.1. Rheology measurements	66
3.3.2.2. Growth factors release assessment	66
3.3.2.2.1. Transforming growth factor-β1 (TGF-β1)	
release assessment	67
3.3.2.2.2. Platelet derived growth factor-BB (PDGF-BB)	
release assessment	68
3.3.2.2.3. Insulin growth factor-1(IGF-1) release	
assessment	69
3.4. Stem cells preparation	71
3.4.1. Stem cells separation and propagation	71
3.4.2. Stem cells encapsulation and proliferation assay	74
3.4.2.1. Periodontal ligament stem cells encapsulation	74
3.4.2.2. Assessment of cell viability and proliferative	
activity	75
3.5. Statistical Analysis	77
Chapter 4: Results	79
4.1. Thermo-sensitive chitosan hydrogel preparation and	
characterization	79
4.1.1. Chitosan powder deacetylation degree measurement	79

Title	Page
4.1.2. Thermo-sensitive chitosan hydrogel preparation and	
gelation	80
4.1.3. Thermo-sensitive chitosan hydrogel characterization	81
4.1.3.1. Rheology measurements	81
4.1.3.2. Biodegradation rate	83
4.2. Freeze-dried platelet concentrate (FDPC) preparation	86
4.3. Platelet concentrate loading and characterization	86
4.3.1. Rheology measurements.	86
4.3.2. Growth factors release assessment	93
4.3.2.1. Transforming growth factor-β1 (TGF-β1) release	
assessment	93
4.3.2.2. Platelet derived growth factor-BB (PDGF-BB)	
release assessment	97
4.3.2.3. Insulin growth factor-1(IGF-1) release	
assessment	100
4.4. Stem cells preparation	101
4.4.1. Stem cells separation and propagation	101
4.4.2. Stem cells encapsulation and proliferation assay	103
4.4.2.1. Stem cells encapsulation in chitosan hydrogel	103
4.4.2.2. Periodontal stem cells viability and proliferation	
activity	104
Chapter 5: Discussion.	107
Chapter 6: Summary and conclusions.	125
References	129

List of Tables

No.	Title	Page
1	Synonyms for the systematic search keywords.	46
2	Population, intervention and control of the current study.	51
3	Outcomes of the current study.	52
4	Materials and chemicals used in the study.	53
5	Experimental design.	55
6	Groups description.	66
7	MTT assay groups.	76
8	Means (\pm SD) and <i>P</i> -values for comparing viscosity (cP) of chitosan/ β -GP solution of each two successive temperature (°C) points.	82
9	Chitosan/ β -GP solution viscosity (cP) comparison (<i>P</i> -values) between the different temperature (°C) points.	83
10	Mean remaining weight percentages, mean weight loss percentages, standard deviations and <i>P</i> -values for comparing each two successive time points of chitosan hydrogel degradation test.	84
11	Comparison between viscosities (cP) at different temperatures (°C) in the four study groups.	88
12	Intra-group viscosity (cP) comparison for group II between the different temperature (°C) points.	89
13	Intra-group viscosity (cP) comparison for group III between the different temperature (°C) points.	90