

**CHITOSAN NANOSCAFFOLD INJECTABLE GEL: A UNIQUE BONE
GLUE BIONANOCOMPOSITES**

Noppadol Trirong

A Thesis Submitted in Partial Fulfilment of the Requirements
for the Degree of Master of Science
The Petroleum and Petrochemical College, Chulalongkorn University
in Academic Partnership with
The University of Michigan, The University of Oklahoma,
and Case Western Reserve University

2012


I26374986

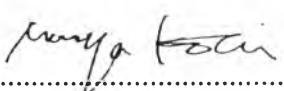
Thesis Title: Chitosan Nanoscaffold Injectable Gel: A Unique Bone Glue
Bionanocomposites
By: Noppadol Trirong
Program: Polymer Science
Thesis Advisors: Prof. Suwabun Chirachanchai

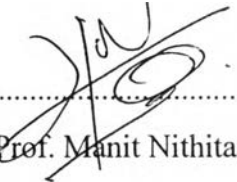
Accepted by The Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfilment of the requirements for the Degree of Master of Science.

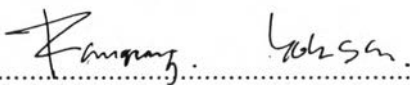

..... College Dean
(Asst. Prof. Pomthong Malakul)

Thesis Committee:


.....
(Prof. Suwabun Chirachanchai)


.....
(Assoc.Prof. Masaya Kotaki)


.....
(Asst. Prof. Manit Nithitanakul)


.....
(Asst. Prof. Rangrong Yongsan)

ABSTRACT

5372012063: Polymer Science Program

Noppadol Trirong: Chitosan Nanoscaffold Injectable Gel: A Unique Bone Glue Bionanocomposites

Thesis Advisor: Prof. Suwabun Chirachanchai, 61 pp.

Keywords: Chitosan nanoscaffold/ Injectable gel/ Carboxymethylchitosan/ Bone glue

Injectable bone glue is a potential material for bone healing due to the possibility of direct injection into bone breaking area, the efficient bone connection through chemical and/ or physical bonds, and the convenient clinical treatment without surgery. Chitosan is a good candidate since it is not only glucosamine via enzymatic degradation, a major component of bone marrow, but also it can be derivatized to highly viscous or gel-type glue. Herein, we propose a novel bone glue bionanocomposite based on chitosan nanoscaffold (CN). The advantages of using CN are the ease of derivatization to be water soluble species (carboxymethylchitosan nanoscaffold, CMCN), the effective heterogeneous reaction to obtain epoxidized CN, and the possibility to use as reinforce material in gel network. The CN gel shows biocompatibility as shown from the SaOs-2 cell culture test. The CN also help the gel incorporation with hydroxyapatite as evidenced from optical microscope and scanning electron microscope (SEM).

บทคัดย่อ

นพดล ไตรรงค์ : ไคโตซาน นาโนสแกฟโฟลด์ แบบเจลชนิด: ไบโอนานาโนคอมโพสิทกาวติดกระดูกแบบลักษณะพิเศษ (Chitosan Nanoscaffold Gel: A Unique Bone Glue Bionanocomposite) อ. ที่ปรึกษา : ศาสตราจารย์ ดร. สุวบุญ จิราญชัย), 61 หน้า

งานวิจัยนี้นำเสนอการติดกระดูกแบบเจล ซึ่งสามารถพัฒนาไปเป็นวัสดุรักษาโรคกระดูกหัก เนื่องจากสามารถฉีดเข้าตรงแนวกระดูกหักได้โดยไม่ต้องผ่าตัด ไคโตซานคือวัสดุที่เหมาะสม สำหรับใช้พัฒนาเป็นกาวรักษากระดูก เมื่อไคโตซานถูกย่อยสลายด้วยเอนไซม์จะให้กลูโคซามีน ซึ่งเป็นส่วนประกอบสำคัญของกระดูก งานนี้ได้พัฒนาไคโตซานไปเป็น กาวติดกระดูกแบบไบโอนานาโนคอมโพสิท โดยใช้ไคโตซานนาโนสแกฟโฟลด์ ที่มีประสิทธิภาพในการเปลี่ยนเป็นอนุพันธ์ที่ละลายน้ำ และสามารถใช้ปฏิกิริยาแบบเนื้อผสมในการเพิ่มหมู่ฟังก์ชันบนพื้นผิว เพื่อเสริมความแข็งแรง เจลชนิดกระดูกนี้มีความสามารถในการช่วยเร่งการเจริญเติบโตของเซลล์ ความสามารถในการเข้ากันได้กับไฮดรอกซีแอปพาไทต์ ซึ่งเป็นส่วนประกอบสำคัญในกระดูก โดยพิสูจน์ได้โดยใช้กล้อง Optical microscope และ Scanning electron microscope (SEM)

ACKNOWLEDGEMENTS

The author would like to express his thanks to his advisor, Professor Suwabun Chirachanchai, who originated this work, gave value suggestions and recommendations, and provided facilities throughout this research. He also would like to acknowledge Asst. Prof. Dr. Sittisak Honsawek for his good recommendations, strong support and his kind.

The author is also thankful to the dissertation committee for their suggestions and recommendations in the reading this thesis book. He greatly appreciates all professors who is his teacher to teach invaluable knowledge to him at The Petroleum and Petrochemical College, Chulalongkorn University.

He would like to thank his seniors at SWB Research Group for their help, invaluable guidance and suggestions throughout this research. He would like to thank the college staff members, and his entire friends at The Petroleum and Petrochemical College, Chulalongkorn University.

This thesis work is funded by the Petroleum and Petrochemical College; and by the Center of Excellence on Petrochemicals, and Materials Technology, Thailand.

Finally, he wishes to express his thank to his family for their support, encouragement, and for being a constant source of his inspiration.

TABLE OF CONTENTS

	PAGE	
Title Page	i	
Abstract (in English)	ii	
Abstract (in Thai)	iii	
Acknowledgements	iv	
Table of Contents	v	
List of Tables	viii	
List of Figures	ix	
List of Scheme	xi	
 CHAPTER		
I	INTRODUCTION	1
II	LITERATURE REVIEW	3
	2.1 General Bone Fracture Healing	3
	2.2 Advanced Bone tissue Scaffold	3
	2.3 Chitosan: Biopolymer for Tissue Engineering	5
	2.4 Chitosan-based Hydrogels	8
	2.5 Injectable Gel	10
	2.6 Bone Morphogenetic Proteins (BMPs)	11
	2.7 Our Idea on the Present Project	11
III	EXPERIMENTAL	13
	3.1 Materials	13
	3.2 Instrument and Equipment	13
	3.3 Methodology	14

CHAPTER	PAGE
IV RESULTS AND DISCUSSION	21
4.1 Abstract	21
4.2 Introduction	22
4.3 Eperimental	24
4.3.1 Materials	24
4.3.2 Instrument and Equipment	24
4.3.3 Preparation of Chitosan Nanoscaffold	25
4.3.4 Synthesis of Carboxymethylchitosan Nanoscaffold	26
4.3.5 Synthesis of Epoxy-chitosan Nanoscaffold	27
4.3.6 Preparation of CMCN Gel	27
4.3.7 Preparation of CMCNB Gel	28
4.3.8 Preparation of CMCNE Gel	28
4.3.9 Swelling Measurement	28
4.3.10 Network Parameters	29
4.3.11 Morphology of Injectable Gel	30
4.3.12 Rheological Analysis	30
4.3.13 Hydroxyapatite Cooperation	31
4.3.14 Cell Culture Studies	31
4.4 Results and Discussions	32
4.4.1 Structural Characterization	32
4.4.2 Solubility of CMCN	36
4.4.3 Gel Formation Studies	37
4.4.4 Swelling Characteristics	39
4.4.5 Network Parameters	39
4.4.6 Morphology Observation	42
4.4.7 Rheological Analysis	43
4.4.8 Mineralization with Hydroxyapatite on Injectable Gel	45
4.4.9 Cell Culture Studies	47
4.4.10 Conclusions	48

CHAPTER		PAGE
IV		
	4.4 10 References	48
V	CONCLUSIONS AND RECOMMENDATIONS	51
	REFERENCES	52
	APPENDIX	57
	Appendix A DNA Assay	57
	Appendix B Drying Technique (Freeze-dry)	59
	CURRICULUM VITAE	60

LIST OF TABLES

TABLE		PAGE
4.1	Water solubility of CMCN in different concentrations and pHs	37
4.2	Gel formation under various concentrations of CMCN and amount of EPC	37
4.3	Equilibrium water content (EWC) and diffusion characteristics	41
4.4	Network parameters of injectable gel	42

LIST OF FIGURES

FIGURE	PAGE
2.1 SEM micrographs of cross-sectional cut of the mineralized scaffold	5
2.2 SEM images of cross-sectional cut hydrogel formed triblock	8
2.3 Injectable gel for broken bone treatment	10
4.1 TEM micrographs of (a) CW, and (b) CN	23
4.2 FTIR spectra of (a) CW, (b) CN, (c) ECN, (d) CMCN, (e) CMCN gel, (f) CMCNB gel, and (h) CMCNE gel	33
4.3 Curve fitting FTIR spectra of (A) ECN and (B) CMCNE gel	34
4.4 ¹ H-NMR spectra of (a) CN in 2%CD ₃ COOD/D ₂ O at 65 °C and (b) CMCN in D ₂ O at 25 °C	35
4.5 Gelation time of CMCN gel from test tube inverting method.	38
4.6 Injectable gel for bone glue material	38
4.7 Percent swelling	40
4.8 TEM micrographs of (a) CN, and (b) ECN	43
4.9 Optical micrographs (left) and SEM micrograph (right) of (A) CMCN-10 gel, (B) CMCNB-10 gel, and (C) CMCNE-10 gel	44
4.10 Storage modulus (G') of CMCN-10 (◆), CMCNB-10 (▲) and CMCNE-10 (■) gel and loss modulus (G'') of CMCN-10 (□), CMCNB-10 (Δ) and CMCNE-10 (◻) gel.	44
4.11 Three types of gels, (A) CMCN-10, (B) CMCNB-10, and (C) CMCNE-10 gel, left hand original gel and right hand gel after mineralization with hydroxyapatite for 3 times.	45
4.12 Percent ash content of (◆) CMCN-10, (■) CMCNB-10, and (▲) CMCNE-10 gel by alternate soaking.	46

LIST OF FIGURES

FIGURE		PAGE
4.13	Optical micrographs of HA formation of, (A) CMCN-10 gel, (B) CMCNB-10 gel, and (C) CMCNE-10 gel.	46
4.14	SEM micrographs of HA formation of, A) CMCN-10 gel, (B) CMCNB-10 gel, and (C) CMCNE-10 gel.	47
4.15	Number of SaOs-2 cells from DNA assays of (■) CMCN-10, (■) CMCNB-10, and (■) CMCNE-10 gel.	48

LIST OF SCHEMES

SCHEME	PAGE
2.1 Chemical structure of chitosan	5
2.2 Chitosan complexation with metal ions	6
2.3 Chemical modifications to obtained water-based chitosan	9
3.1 Preparation of chitosan nanoscaffold	15
3.2 Synthesis of carboxymethylchitosan nanoscaffold	16
3.3 Synthesis of epoxy-chitosan nanoscaffold	16
4.1 Preparation of chitosan nanoscaffold	25
4.2 Synthesis of carboxymethylchitosan nanoscaffold	27
4.3 Synthesis of epoxy-chitosan nanoscaffold	27
4.4 Mechanism of CMCN gel	32
4.5 Mechanism of gel formation of CMCNE gel	36