BIOACTIVE POLY(Ɛ-CAPROLACTONE) SCAFFOLDS FROM PICKERING EMULSION TEMPLATING

MEENAL AGRAWAL

DEPARTMENT OF TEXTILE AND FIBRE ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI
MARCH 2026

Bioactive Poly(ε-caprolactone) Scaffolds from Pickering Emulsion Templating

by

Meenal Agrawal

Department of Textile and Fibre Engineering

Submitted
in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the

Indian Institute of Technology Delhi

March 2026

|| सा विद्या या विमुक्तये ||

Dedicated to

My father
“Mr. Vijay Agrawal”

&

My mother
“Mrs. Rekha Agrawal”

Certificate

This is to certify that the thesis titled “**Bioactive Poly(ε-caprolactone) Scaffolds from Pickering Emulsion Templating**”, being submitted by **Ms. Meenal Agrawal** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her. She has worked under my guidance and supervision and fulfilled the requirements for submission of the thesis which has attained the standard required for a Ph. D. degree of this Institute.

The results contained in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

Prof. Rajiv K. Srivastava
Department of Textile and Fibre Engineering
Indian Institute of Technology Delhi
New Delhi – 110016, India

Date:

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisor, Prof. Rajiv K. Srivastava, for providing me with the opportunity to pursue my PhD research under his mentorship. His invaluable guidance, constant encouragement, and unwavering support, combined with his deep scientific insight, meticulous approach, and critical evaluation, has significantly shaped the direction of this thesis and helped me overcome numerous research challenges while refining my understanding at every stage of the work. I am especially grateful for his patience and approachability, which has created a supportive environment that encouraged open discussions and independent thinking. Through his mentorship, I learned not only advanced scientific concepts and experimental rigor but also the importance of perseverance, ethical research practices, and critical problem-solving. His mentorship has not only contributed to my academic growth but has also transformed me into a disciplined and independent scientific researcher, for which I will always remain deeply thankful.

I would like to thank the members of my Student Research Committee Prof. Bhupendra Singh Butola, Prof. Bhanu Nandan, and Prof. Bhabani K. Satapathy for their periodic review of my work and for providing valuable suggestions at regular intervals. Their constructive feedback and critical insights helped in refining the direction, quality, and clarity of this research.

I also take this opportunity to acknowledge Department of Textile and Fibre Engineering along with the present and all former Head of the Department- Prof. Deepti Gupta, Prof. Ramasamy Alagirusamy, Prof. R. S. Rengasamy, and Prof. Ashwini Kumar Agrawal for continuously upgrading the department with advanced research facilities and improved infrastructure, which has created a conducive and enriching environment for research and learning. I also grateful to all the technical personnel and office staff of the department as well as institute for their assistance and cooperation during my PhD tenure.

I am grateful to my collaborators Prof. Ritu Kulshreshtha and Prof. Sachin Kumar for their assistance and expertise towards the biological characterization of my samples, which greatly strengthened the findings of this work.

I sincerely thank Dr. Anilkumar L. Yadav for mentoring me during my tenure and for teaching me how to effectively plan and execute research and experiments, as well as for his guidance in scientific writing and manuscript preparation. His practical insights and structured approach have been invaluable to my research training.

I appreciate my current and former lab mates Dr. Pramod M. Gurave, Dr. Dheeraj Kumar, Dr. Sweety Rani, Dr. Advitiya Kumar, Dr. Rosemary Thomas, Dr. Sajan Singh, Dr. Avinash Raulo, Dr. Sumana Bandyopadhyay, Dr. Aashish Joshi, Dr. Ekta Vashishth, Dr. Puhup Puneet, Mr. Sagnik Ghosh, Mr. Shubhang Dubey, Mr. Sachidananda Mohapatra, Ms. Vanshika Handuja, Mr. Arvind Kumar, Ms. Bhagyashree Bhattacharyya, Ms. Smriti Mishra, Ms. Saptaparni Chanda, Mr. Sagar Singh, Mr. Ashish Mishra, Mr. Anil Yadav, Mr. Ashish Yadav, Ms. Juviya Mathew, Mr. Soumik Kumar, Mr. Subarna Halder, and Ms. Sristi Katiyar for their constant support throughout my research. Their assistance during experiments, constructive discussions, and willingness to share knowledge created a positive and motivating environment. A special mention to my mentee Ms. Twinkle Suneja, Mr. Faiz Alam and Mr. Mohammad Sajedin for their sincere efforts, cooperation, and dedication during the work.

I also acknowledge my friends from IIT Delhi Ms. Mamta, Dr. Rupali, Dr. Gurneet, Akash, Annwesha, Nilotpal, Rohini, Rukmini, Tejal, Suchita, Bharti, Srishti, Kanchan, Achala, Meghana, Samidha, Doyel, Kavya, Sovan, Chandrashish, and Ashmit for their encouragement, timely distractions, and much-needed breaks from research which helped me navigate the inevitable highs and lows of doctoral life, making the journey both enjoyable and memorable.

I cannot fail to mention Dr. Manali, Mr. Porav, Ms. Komal, Ms. Monika, and Ms. Devyani who stood by me throughout the journey. Their honesty, humour, and unwavering support provided the balance and perspective during challenges and celebrations. My heartfelt regards to Mrs. Vaneshwari Mulgund, the first teacher and mentor of my life. It was under her patient guidance that I first learned to read and write, lessons that became the foundation of my future learning.

I owe my deepest gratitude to my parents, Mr. Vijay Agrawal and Mrs. Rekha Agrawal, for their unwavering love, guidance, and countless sacrifices, which have been the foundation of my growth, values, and achievements. Their constant encouragement and belief in my abilities have shaped me into the person I am today. To my brother, Mr. Prateek Agarwal, thank you for always keeping me grounded, and for being both a critic and a cheerleader in your own unique way. I am also profoundly grateful to my grandparents, Late. Mr. Raghuveer Prasad Agrawal and Mrs. Kamlesh Devi Agrawal, for their wisdom, blessings, and constant faith in me.

Last but not the least, I bow in gratitude to the Almighty for providing me with strength, positivity, and self-belief throughout this journey.

Meenal Agrawal

Abstract

While bone tissues have regenerative capabilities to cure minor injuries, the process is inefficient for large sized bone defects and hence requires clinical interventions. Conventional treatments like autografts and allografts are still considered as the gold standard to cure the condition, however they present severe challenges due to their limited availability along with post-operative complications. To overcome these challenges, scaffolds are being developed as support structures that enable cell adhesion, proliferation, and subsequent tissue regeneration. Achieving optimal mechanical stability and bioactivity relies on careful control of scaffold design, which is governed by material choice, fabrication technique, and post-fabrication modifications. For this, high internal phase emulsion (HIPE) templating is of huge importance considering its capability to produce highly porous three-dimensional scaffolds. While HIPE templating has been studied for wide range of polymers, poly(ε-caprolactone) (PCL) based HIPE templated constructs are most widely used for bone tissue regeneration. However, PCL based HIPE templating can require very high concentration of surfactants (up to 60 wt%) which not only requires rigorous purification process but also generate environmental waste.

To address this, we have developed surfactant-free Pickering HIPE templating of ε-caprolactone (CL) using hydrophobically modified fumed silica nanoparticles (mSiNP) as stabilizers. The resulting Pickering HIPE demonstrated excellent stability further polymerizing at 120 °C. The physicochemical properties of the scaffolds were thoroughly characterized to confirm their chemical and mechanical compatibility at the implant-site. An antimicrobial therapeutic, clove oil (CO), was used to impart antimicrobial properties within scaffolds which were evaluated against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. The cytocompatibility of CO-incorporated scaffolds was confirmed using MG-63 cells over the period of 7 days by observing the metabolic activity of cells in the presence of pristine and CO-incorporated scaffolds.

A detailed thermal analysis of the scaffolds was carried out to study the effect of crosslinking, mSiNP, and porosity on the crystallization behaviour of the scaffolds. The porosity of the scaffolds was controlled by adjusting the dispersed phase volume fraction (ϕ_d) of HIPE. The thermal behaviour was observed under non-isothermal conditions, and the resulting curves were analyzed using Jeziorny, Ozawa, and Mo models. While crosslinking and mSiNP led to retarded crystallization kinetics due to hindered chain mobility, the effect was further elevated in the presence of porosity. The x-ray diffraction studies also confirmed significant changes in the crystalline structure of the polymer at very high porosity.

The use of functional initiator for the polymerization of PCL was explored using hydroxyethyl cellulose (HEC). The unreacted hydroxyl groups of HEC not only controlled the hydrophobicity of the scaffolds but also provided functional sites for surface modification. Type 1 collagen was incorporated over the scaffolds using a combination of Malaprade oxidation and Schiff base chemistry utilizing the primary amine groups in collagen. The cytocompatibility of collagen functionalized scaffolds was confirmed using MG-63 cells wherein the presence of collagen not only led to higher number of metabolic cells over the surface of scaffolds but also promoted improved cell adhesion due to its bioactive sites.

Photocurable PCL oligomers were also synthesized using hydroxyethyl methacrylate (HEMA) as an initiator for PCL. The resulting PCL oligomers were HIPE templated and photocured to form crosslinked porous scaffolds using ethylene glycol dimethacrylate (EGDMA). The effect of initiator concentration, comonomer concentration, and ϕ_d on physicochemical properties of the polymer and scaffolds was also studied. The resulting porous scaffolds were incorporated with tea tree oil (TTO) and the sustained release kinetics of TTO was elaborated using Weibull and Ritger-Peppas models. The TTO-loaded scaffolds demonstrated excellent antimicrobial properties against *S. aureus* and *E. coli* confirming their potential as antimicrobial therapeutic delivery carriers.

सारांश

हड्डी के ऊतकों में मामूली चोटों को ठीक करने की स्वाभाविक पुनर्जनन क्षमता होती है, लेकिन बड़े आकार की हड्डी की क्षति के लिए यह प्रक्रिया पर्याप्त नहीं होती और इसलिए चिकित्सीय हस्तक्षेप की आवश्यकता पड़ती है। परंपरागत उपचार जैसे स्व-प्रतिरोपण और पर-प्रतिरोपण अभी भी इस स्थिति के उपचार के लिए स्वर्ण-मानक माने जाते हैं, लेकिन इनकी सीमित उपलब्धता तथा शल्यचिकित्सा के बाद होने वाली जटिलताओं के कारण ये चिकित्सा क्षेत्र के लिए गंभीर चुनौतियाँ प्रस्तुत करते हैं। इन चुनौतियों को दूर करने के लिए, ऐसे स्कैफोल्ड विकसित किए जा रहे हैं जो कोशिकाओं के आसंजन, वृद्धि और बाद में ऊतक पुनर्जनन को सक्षम बनाने वाले सहायक ढाँचे का कार्य करते हैं। स्कैफोल्ड के यांत्रिक स्थायित्व और जैव-सक्रियता को बेहतर बनाए रखने के लिए उसके अभिकल्पना पर सटीक नियंत्रण आवश्यक है, जो उपयोग की गई सामग्री, निर्माण तकनीक और निर्माण के बाद किए गए संशोधनों से प्रभावित होता है।

इस उद्देश्य के लिए, हाई इंटरनल फेज इमल्शन (HIPE) टेम्पलेटिंग अत्यधिक छिद्रयुक्त, त्रि-आयामी स्कैफोल्ड तैयार करने की अपनी क्षमता के कारण अत्यंत महत्वपूर्ण है। यद्यपि HIPE टेम्पलेटिंग विभिन्न प्रकार के पॉलिमरों के लिए अध्ययन की गई है, पॉली (ε-कैप्रोलैक्टोन) (PCL) आधारित HIPE टेम्पलेटेड स्कैफोल्ड हड्डी ऊतक पुनर्जनन के लिए सबसे अधिक उपयोग किए जाते हैं। हालांकि, PCL आधारित HIPE टेम्पलेटिंग में बहुत अधिक मात्रा में सर्फैक्टेंट (60 wt% तक) की आवश्यकता हो सकती है, जिससे कठोर शुद्धिकरण प्रक्रियाओं की आवश्यकता पड़ती है तथा पर्यावरणीय अपशिष्ट भी उत्पन्न होता है।

इस समस्या को हल करने के लिए, हमने हाइड्रोफोबिक रूप से संशोधित फ्यूम्ड सिलिका नैनोकणों (mSiNP) को स्थिरकारक के रूप में उपयोग करते हुए ε-कैप्रोलैक्टोन (CL) की सर्फैक्टेंट-रहित पिकरिंग HIPE टेम्पलेटिंग विकसित की है। प्राप्त पिकरिंग HIPE ने उत्कृष्ट स्थिरता प्रदर्शित की और 120°C पर सफलतापूर्वक पॉलिमराइज़ हुई। स्कैफोल्ड की भौतिक-रासायनिक विशेषताओं का व्यापक विश्लेषण किया गया ताकि इम्प्लांट साइट पर उनकी रासायनिक तथा यांत्रिक अनुकूलता की पुष्टि की जा सके। एंटीमाइक्रोबियल उपचारक के रूप में लौंग का तेल (CO) स्कैफोल्ड में सम्मिलित किया गया, जिसने ग्राम-पॉज़िटिव (*S. aureus*) और ग्राम-नेगेटिव (*E. coli*) बैक्टीरिया के विरुद्ध उत्कृष्ट रोगाणुरोधी गुण प्रदर्शित किए। CO-संलक्षित स्कैफोल्ड की साइटोकम्पैटिबिलिटी MG-63 कोशिकाओं पर 7 दिनों की अवधि में कोशिका चयापचय गतिविधि का परीक्षण करके सत्यापित की गई।

स्कैफोल्ड की थर्मल विशेषताओं का विस्तृत विश्लेषण किया गया, ताकि क्रॉसलिंकिंग, mSiNP तथा छिद्रता का पॉलिमर के क्रिस्टलीकरण व्यवहार पर प्रभाव समझा जा सके। स्कैफोल्ड की छिद्रता को HIPE के डिस्पर्स फेज वॉल्यूम फ्रैक्शन (ϕ_d) को समायोजित करके नियंत्रित किया गया। थर्मल व्यवहार को नॉन-आइसोथर्मल परिस्थितियों में देखा गया तथा परिणामों का विश्लेषण Jeziorny, Ozawa और Mo मॉडल का उपयोग करके किया गया। क्रॉसलिंकिंग और mSiNP ने श्रृंखला गतिशीलता में कमी के कारण क्रिस्टलीकरण गतिशीलता को धीमा किया, और यह प्रभाव उच्च छिद्रता की उपस्थिति में और अधिक बढ़ गया। एक्स-रे विवर्तन (XRD) अध्ययन ने भी बहुत अधिक छिद्रता पर पॉलिमर की क्रिस्टलीय संरचना में महत्वपूर्ण परिवर्तन की पुष्टि की।

PCL की पॉलिमराइज़ेशन के लिए कार्यात्मक इनिशिएटर के रूप में हाइड्रॉक्सीएथाइल सेल्युलोज़ (HEC) के उपयोग का अध्ययन किया गया। HEC के अपरिक्रियाशील हाइड्रॉक्सिल समूहों ने न केवल स्कैफोल्ड की हाइड्रोफोबिसिटी को नियंत्रित किया, बल्कि सतही संशोधन के लिए कार्यात्मक स्थलों की भी उपलब्धता प्रदान की। टाइप 1 कोलेजन को मालाप्राड ऑक्सीकरण और शिफ़ बेस रसायन के संयोजन का उपयोग करके स्कैफोल्ड पर सम्मिलित किया गया, जिसमें कोलेजन के प्राथमिक अमीन समूहों का उपयोग किया गया। कोलेजन-संशोधित स्कैफोल्ड की साइटोकम्पैटिबिलिटी MG-63 कोशिकाओं पर की गई, जिसमें कोलेजन की उपस्थिति ने स्कैफोल्ड सतह पर अधिक चयापचयी सक्रिय कोशिकाओं के निर्माण के साथ-साथ उसकी जैव-सक्रिय साइटों के कारण बेहतर कोशिका आसंजन भी दर्शाया।

फोटोक्युरेबल PCL ओलिगोमर्स का संश्लेषण हाइड्रॉक्सीएथिल मेथाक्रिलेट (HEMA) को PCL के इनिशिएटर के रूप में उपयोग करके किया गया। प्राप्त PCL ओलिगोमर्स को HIPE टेम्पलेट किया गया और एथिलीन ग्लाइकोल डाइमेथाक्रिलेट (EGDMA) का उपयोग करके फोटोक्योर कर क्रॉसलिंक्ड छिद्रयुक्त स्कैफोल्ड में परिवर्तित किया गया। इनिशिएटर सांद्रता, को-मोनोमर सांद्रता तथा ϕd का पॉलिमर और स्कैफोल्ड की भौतिक-रासायनिक विशेषताओं पर प्रभाव भी अध्ययन किया गया। अंतिम छिद्रयुक्त स्कैफोल्ड में टी ट्री ऑयल (TTO) को सम्मिलित किया गया, और इसके सतत रिलीज़ गतिशीलता का विश्लेषण वेइबुल और रिटगर–पेप्पास मॉडलों का उपयोग करके किया गया। TTO-संयोजित स्कैफोल्ड ने *S. aureus* और *E. coli* के विरुद्ध उत्कृष्ट रोगाणुरोधी गुण प्रदर्शित किए, जो इन्हें रोगाणुरोधी उपचारक वितरण वाहक के रूप में उपयोग करने की संभावनाओं की पुष्टि करते हैं।

Table of Contents

Content		Page number
Certificate		i
Acknowledgements		ii
Abstract		iv
सारांश		vi
Table of Contents		viii
List of Figures and Schemes		xiv
List of Tables		xx
List of Symbols		xxi
List of Abbreviations		xxiii
Chapter 1	**Introduction and Motivation**	**1-10**
1.1	Background	2
1.2	Objective of work	4
1.3	Thesis organizational structure	5
1.4	References	9
Chapter 2	**Literature Review**	**11-47**
2.1	High internal phase emulsion (HIPE) templating	12
2.2	Stabilization of HIPE	18
	2.2.1 Surfactant-stabilized HIPE	18
	2.2.2 Particle-stabilized Pickering HIPE	19
	2.2.3 Factors affecting HIPE stability	20
2.3	HIPE templated poly (ε-caprolactone) (PCL) scaffolds	22
2.4	Strategy towards PCL modification for biomedical application	31
	2.4.1 Physical modification of PCL scaffolds	31
	2.4.2 Chemical modification of PCL scaffolds	32
2.5	Biomedical applications of HIPE templated PCL scaffolds	33
2.6	Summary	35
2.7	Research objectives	36
2.8	References	36

Chapter 3 Experimental Techniques 48-57

3.1 Introduction 49

3.2 Materials 49

3.3 Methods

3.3.1 Synthesis of BCY crosslinker 50

3.3.2 Preparation of CL based Pickering high internal phase emulsions (Pickering HIPE) 51

3.3.3 Preparation of PCL-based nanocomposite scaffolds 51

3.3.4 Therapeutic loaded PCL scaffolds 51

3.3.5 Protein immobilization on the surface of scaffold 52

3.3.6 Preparation of phosphate buffer solution (PBS) 52

3.4 Characterization

3.4.1 Nuclear magnetic resonance (NMR) 52

3.4.2 Steady shear analysis 53

3.4.3 Drop shape analysis 53

3.4.4 Optical microscopy 53

3.4.5 Confocal microscopy 53

3.4.6 Scanning electron microscopy (SEM) 53

3.4.7 Porosity 53

3.4.8 Liquid uptake capacity and gel content 54

3.4.9 Cyclic compression test 54

3.4.10 Fourier Transform Infrared Spectroscopy (FTIR) 54

3.4.11 Energy-dispersive X-ray spectroscopy (EDX) 55

3.4.12 Transmission electron microscopy (TEM) 55

3.4.13 X-ray photoelectron spectroscopy (XPS) 55

3.4.14 Differential scanning calorimetry (DSC) 55

3.4.15 X-ray diffraction (XRD) 55

3.4.16 Cumulative release profile of therapeutic 56

3.4.17 Zone of inhibition (ZOI) 56

3.4.18 Bacterial reduction percentage 56

3.4.19 Biofilm adhesion 56

3.4.20 Bacterial morphology 57

3.4.21 Cytoplasmic content 57

3.4.22 MTT assay 57

3.5 References 67

Chapter 4 **Stabilization of Pickering HIPE Leading to the Fabrication of Crosslinked Poly(ε-caprolactone) PolyHIPE with Optimized Stabilizer Concentration** **58-76**

4.1 Introduction 59

4.2 Materials 60

4.3 Procedures

4.3.1 Synthesis of crosslinked PCL nanocomposite polyHIPEs 61

4.3.2 Synthesis of crosslinker BCY 62

4.3.3 Characterization 62

4.4 Results and discussions

4.4.1 Stabilization of PCL based Pickering HIPE 65

4.4.2 Formation of porous nanocomposite PCL polyHIPE 66

4.4.3 Physicochemical characterization of polyHIPE 68

4.4.4 Thermal characterization of PCL polyHIPE 69

4.4.5 Liquid uptake capacity of PCL polyHIPE 70

4.4.6 Optimization of stabilizer concentration for PCL polyHIPE synthesis 72

4.5 Conclusions 74

4.6 References 75

Chapter 5 **Development of Pickering HIPE Templated Poly(ε-caprolactone) Scaffolds with Antimicrobial Properties for Bone Regeneration** **77-103**

5.1 Introduction 78

5.2 Materials 79

5.3 Procedures

5.3.1 Preparation of cPCL nanocomposites via Pickering HIPE-ROP 80

5.3.2 Preparation of clove oil (CO)-incorporated cPCL nanocomposite scaffolds 81

5.3.3 Preparation of phosphate buffer solution (PBS) 81

5.3.4 Characterization 81

5.4 Results and discussions

5.4.1 Stabilization of Pickering HIPEs 85
5.4.2 cPCL nanocomposite scaffolds 88
5.4.3 Mechanical properties of scaffolds 90
5.4.4 Liquid uptake of scaffolds 90
5.4.5 Clove oil (CO)- incorporated scaffolds 92
5.4.6 Release kinetics 94
5.4.7 Antimicrobial properties of the scaffolds 94
5.4.8 Bacterial morphology 95
5.4.9 Biofilm inhibition 97
5.4.10 Cell viability 98
5.5 Conclusions 100
5.6 References 100
Chapter 6 Thermal Behaviour of Poly(ε-caprolactone) Scaffolds as a Function of Porosity 104-127
6.1 Introduction 105
6.2 Materials 106

6.3 Procedures

6.3.1 Synthesis of non-porous poly(ε-caprolactone) (PCL) 106
6.3.2 Synthesis of emulsion templated porous PCL scaffolds 107
6.3.3 Characterization 108

6.4 Results and discussions

6.4.1 Stabilization of Pickering emulsions 110
6.4.2 Fabrication of nanocomposite scaffolds 111
6.4.3 Thermal behavior of non-porous and porous scaffolds 112
6.4.4 Crystallization kinetics of non-porous and porous scaffolds 115
6.4.5 Jeziorny model 116
6.4.6 Ozawa model 118
6.4.7 Mo model 120
6.4.8 Nucleation energy 122
6.4.9 Crystalline structure of non-porous and porous scaffolds 123

6.5 Conclusion 124
6.6 References 125
Chapter 7 Utilization of Functional Initiator for Poly(ε-caprolactone) Polymerization to Facilitate Surface Functionalization of Scaffolds 128-154
7.1 Introduction 129
7.2 Materials 131
7.3 Procedure
  7.3.1 Preparation of PCL nanocomposite scaffolds via Pickering HIPE-ROP 131
  7.3.2 Collagen functionalization of scaffolds 132
  7.3.3 Preparation of phosphate buffer solution (PBS) 132
  7.3.4 Characterization 132
7.4 Results and discussions
  7.4.1 Fabrication of HEC-g-PCL scaffolds 138
  7.4.2 Physicochemical and mechanical characterization of HEC-g-PCL scaffolds 141
  7.4.3 Functionalization of HEC-g-PCL scaffolds with type I collagen 145
  7.4.4 Cytocompatibility of collagen functionalized OHEC-g-PCL scaffolds 147
7.5 Conclusions 151
7.6 References 152
Chapter 8 Fabrication of Photocured Poly(ε-caprolactone) PolyHIPEs Using Low Molecular Weight Oligomers 155-182
8.1 Introduction 156
8.2 Materials 157
8.3 Procedures
  8.3.1 Synthesis of photocurable PCL oligomers 158
  8.3.2 Crosslinking of photocurable PCL oligomers 158
  8.3.3 HIPE-templated photocured crosslinked PCL drug carriers 158

8.3.4 Incorporation of TTO in the porous crosslinked PCL polyHIPEs 160
8.3.5 Preparation of PBS 160
8.3.6 Characterization 160

8.4 Results and discussions

8.4.1 Synthesis of photocurable PCL oligomers 164
8.4.2 Fabrication of photo-crosslinked PCL polyHIPEs 167
8.4.3 TTO-loaded porous polyHIPEs 171
8.4.4 TTO release kinetics from TTO-loaded porous polyHIPEs 172
8.4.5 Antimicrobial properties of TTO-loaded porous polyHIPEs 175
8.4.6 Bacterial killing mechanism 176
8.4.7 Biofilm formation 177
8.5 Conclusions 179
8.6 References 180
Chapter 9 Conclusions and Future Outlook 183-187
9.1 Conclusions 184
9.2 Future outlook 186
Curriculum Vitae 188-190

List of Figures and Schemes

Figure number	Figure caption	Page number
2.1	Schematic representation for fabrication of 3D porous scaffold using HIPE templating.	17
3.1	Schematic representation of the procedure for the fabrication of PCL based nanocomposite scaffolds through Pickering HIPE templating.	52
4.1	Stabilization of Pickering HIPE using mSiNP at various stabilizer concentration and ϕ_d. (A) Digital images of Pickering HIPE stabilized using various clay as Pickering stabilizer. (B) Digital image of Pickering HIPE of CL stabilized using mSiNP as Pickering stabilizer. (C) TEM image of mSiNP used to stabilize CL based Pickering HIPE. (B) Optical images of Pickering HIPEs stabilized using mSiNP at varied concentration and ϕ_d.	66
4.2	SEM image of C5V75 (inset showing the surface roughness) and C5V80 polyHIPEs.	67
4.3	FTIR spectra of neat PCL and C5V75.	68
4.4	Stress *vs.* strain curve for C5V75.	69
4.5	DSC thermograms of C5V75 Pickering polyHIPE recorded at 10 °C/min rate.	70
4.6	(A) Chloroform sorption values of C5V75 polyHIPE during cyclic sorption-desorption study. (B) Dimensional changes at equilibrium chloroform sorption by C5V75 polyHIPE.	71
4.7	SEM images of polyHIPEs fabricated using mSiNP (5 wt.%) and surfactant Pluronic F127 (0-10 wt.%) as co-stabilizers.	71
4.8	(A) SEM image of polyHIPE with both unmodified SiNP and mSiNP in continuous phase. (B) SEM image of polyHIPE fabricated using unmodified SiNP in continuous phase and mSiNP in dispersed phase. (C) SEM image of resulting polyHIPE with mSiNP in both continuous and dispersed phase. (D) SEM images of polyHIPEs fabricated using mSiNP in only dispersed phase at various mSiNP concentration and ϕ_d.	72
5.1	(A) Contact angle of CL and silicone oil with respect to mSiNP. (B) Digital images of the interface over a period of time when mSiNP was dispersed in different phases. (C) Graphical representation of the concentration required to stabilize Pickering HIPEs when mSiNP was dispersed in CL and silicone oil. (D) Digital images of Pickering HIPEs stabilized at room temperature and 120 °C. Arrows indicate the phase	89

separation in the HIPEs. (E) Apparent viscosity of CL at varied shear rates before and after 10 min of polymerization. (F) Optical images of Pickering HIPEs formulated at varied mSiNP concentration and ϕ_d.

5.2 (A) SEM images of nanocomposite Pickering cPCL scaffolds. (B) Graphical summary of droplet and pore sizes of various Pickering HIPEs and polyHIPE scaffolds, respectively. (C) FTIR graphs of nanocomposites fabricated at varied mSiNP concentration. (D) Compressive stress-strain curves of the scaffolds fabricated with different mSiNP loadings. 91

5.3 Liquid uptake capacity of various cPCL scaffolds in chloroform and PBS. 92

5.4 (A) FTIR graphs of cPCL scaffolds incorporated with varied loading of CO. (B) Compressive stress-strain curves of various CO-incorporated scaffolds. (C) Cumulative release profile of CO from the fabricated C5 nanocomposite scaffold. Inset shows the detailed release profile of CO during initial 12 h. 93

5.5 (A) Digital images of ZOI against S. aureus and E. coli resulting from various CO-incorporated scaffolds. (B) Quantitative representation of resulting ZOI. (C) Bacterial reduction percentage due to various CO-incorporated scaffolds against S. aureus and E. coli. (D) Digital images of the CFUs supported by various scaffolds. 96

5.6 (A) Bacterial morphology observed using TEM when treated with pristine and C5 scaffolds. (B) SEM images of the bacteria post-treatment with pristine and C5 scaffolds. (C) UV absorbance due to nucleic acid extravasated from S. aureus and E. coli resulting from treatment with pristine and C5 scaffolds. 97

5.7 Biofilm formed by (A) S. aureus and (B) E. coli on the surface of scaffolds when treated with pristine and C5 scaffolds. 98

5.8 (A) Viability of MG-63 cells seeded on pristine and C5 scaffolds. (B) Morphology of MG-63 cell adhered on C5 scaffold after the incubation of 24 h. (C) SEM image of the C5 scaffold seeded with MG-63 cells and incubated for 7 days. Inset shows mineralization of the scaffold due to ECM deposition. 99

6.1 Optical images of various precursor solutions and Pickering emulsions of CL stabilized using mSiNP at ϕ_d varied between 0.55-0.85. 110

6.2 SEM images of various non-porous and porous scaffolds resulting from the polymerization of corresponding precursor solutions and Pickering emulsions. 111

6.3 (A) High magnification SEM image demonstrating the surface roughness of resulting PCL Pickering polyHIPEs due to the inclusion of 112 mSiNP. (B) EDX image of PCL Pickering polyHIPEs demonstrating the even presence of Si, confirming the presence of mSiNP.

6.4 DSC thermograms of various non-porous and porous scaffolds resulting from (A) cooling cycle after melting at 10 °C/min, and subsequent (B) 2nd melting at 10 °C/min. (C) the resulting X_{cd} of various scaffolds. (D) Schematic illustration of the effect of increase in ϕ_d on the interfacial thickness and confinement within the porous scaffolds. 114

6.5 Relative crystallinity of various non-porous and porous scaffolds with respect to (A) temperature and (B) time estimated at 10 °C/min cooling rate. (C) The resulting Avrami plots between $\ln[-\ln(1 - X_t)]$ and $\ln t$ for various scaffolds. 118

6.6 Crystallization and 2nd melting curves of various samples recorded at (A) 2 °C/min, (B) 18 °C/min, and (C) 26 °C/min. 119

6.7 Ozawa plots for various non-porous and porous scaffolds demonstrating the relation between X_t and β at given temperatures. 120

6.8 Mo plots for various non-porous and porous scaffolds demonstrating the relation between $\ln t$ and $\ln\beta$ at given extent of crystallinity. 121

6.9 (A) XRD patterns demonstrating the crystalline structures of various non-porous and porous scaffolds, recorded at their crystallization temperatures. (B) XRD pattern of various non-porous and porous scaffolds between 18° and 30°. (C) Deconvoluted peaks of XRD scans demonstrating the crystalline peaks and amorphous curve of various non-porous and porous scaffolds. 124

7.1 Schematic representation illustration the preparation of HEC-g-PCL nanocomposite scaffolds further functionalized with type 1 collagen using the combination of Malaprade oxidation and Schiff base reaction. 132

7.2 Various HIPE templated PCL scaffolds synthesized using HEC as a macroinitiator for polymerization of PCL. (A) Chemical scheme demonstrating HEC as a macro-initiator for polymerization of CL and BCY leading to the formation of crosslinked HEC-g-PCL polymer network. (B) Digital image confirming the formation of uniform solution of HEC in CL at 2 wt% concentration prepared at 120 °C. (C) Apparent viscosity of various HEC and CL solutions at varied shear rate illustrating the effect of HEC concentration on rheological properties of the solution. (D) Fluorescence microscopic images exhibiting the droplet morphology of various Pickering HIPE prepared using mSiNP as Pickering stabilizer with varied HEC concentration. (E) SEM images capturing the pore morphology of various scaffolds fabricated by polymerization and purification of corresponding Pickering HIPE. 140

7.3 Physicochemical and mechanical properties of PCL scaffolds synthesized using HEC as a macroinitiator. (A) Contact angle of various copolymers fabricated at varied HEC concentration confirming its effect on hydrophilicity of the material. (B) XPS service spectra demonstrating the effect of inclusion of HEC on its O/C ratio. (C) C 1s peaks of various scaffolds illustrating the presence of unreacted -OH groups in HEC1.0 scaffold. (D) EDX elemental mapping of HEC1.0 scaffold illustrating the uniform presence of Si corresponding to mSiNP throughout the surface. (E) Liquid uptake capacity of the scaffolds in hydrophobic (DCM) and hydrophilic (PBS) media. (F) Cyclic compressional curves of the scaffolds analyzed up to 60% strain for 5 cycles. 143

7.4 Surface functionalization of HIPE templated PCL scaffolds using type 1 collagen. (A) Chemical schematic illustrating the functionalization of HEC-g-PCL scaffolds with type 1 collagen using a combination of Malaprade oxidation and Schiff base chemistry. (B) FTIR spectra of various scaffolds demonstrating the change in peaks owing to collagen functionalization over the surface of scaffolds. (C) XPS spectra of collagen powder and collagen functionalized OHEC1.0-COL scaffold. The arrow indicates the peak at 400 eV corresponding to N 1s. (D) EDX elemental mapping of OHEC1.0-COL scaffold confirming the uniform presence of N corresponding to collagen type 1 throughout the surface of the scaffolds. (E) Type 1 collagen antibody staining illustrating the uniform presence of type 1 collagen owing to its successful immobilization throughout the surface of OHEC1.0-COL scaffold 146

7.5 Cytocompatibility and osteogenic bioactivity evaluation of developed collagen functionalized PCL-g-OHEC scaffolds. (A) Live-dead staining of MG-63 cells after day 3 and day 7 incubated over the surface of various scaffolds. (B) SEM images demonstrating the cellular adhesion and morphology on the surface of the scaffolds observed after 3 days of incubation. Arrows indicate the porous architecture of the scaffold underneath the layer of cell. (C) The absorbance values observed during MTT assay resulting from the metabolically active cells on the scaffolds evaluated at day 1, 3, and 7. (D) The ALP activity of MG-63 cells assessed after 7 and 14 days of incubation on the scaffolds. 150

8.1 Schematic representation of HIPE templating leading to the formation of TTO-loaded nanocomposite porous polyHIPEs. 159

8.2 Chemical scheme for the preparation of photocurable PCL oligomers that could further polymerize and crosslink using EGDMA in the presence of photoinitiator. 165

8.3 (A) Digital images of HEMA initiated PCL oligomers with varied ratios of CL:HEMA. (B) ^{1}H NMR spectra of monomers and HEMA initiated ROP synthesized PCL oligomers at varied CL:HEMA ratios. (C) FTIR spectra CL, HEMA and HEMA initiated O1 PCL oligomer. (D) Digital images demonstrating the solubility of photocured PCL oligomers with 166 varied crosslinker EGDMA concentrations. The arrows point at undissolved remains of crosslinked PCL. (E) Gel content of O1 PCL oligomers photocured and crosslinked at various EGDMA density. (F) DSC thermograms of O1X0 and O1X5 demonstrating the effect of crosslinking on thermal properties of photocured PCL.

8.4 (A) Confocal images of resulting HIPE formulated using O1 oligomer at various crosslink densities and ϕ_d. (B) SEM images of corresponding porous polyHIPEs resulting from the polymerization of HIPE. The graph demonstrates mean droplet diameter of stable HIPEs and the mean pore size of corresponding polyHIPEs. 168

8.5 (A) Higher magnification SEM image of O1X4-75 demonstrating the roughness at the interface which was attributed to the presence of mSiNP. (B) EDX elemental mapping to detect the presence of C, O, and Si on the surface of polyHIPEs. (C) FTIR spectra of photocurable PCL oligomer, crosslinker, and mSiNP along with the fabricated porous polyHIPE. (D) Compressional mechanical strength of porous polyHIPEs analyzed up to 60% strain. (E) Liquid uptake capacity of the porous polyHIPEs in both hydrophobic (DCM) and hydrophilic (PBS) media. (F) DSC thermograms of O1X4-75 polyHIPE demonstrating both crystallization and 2nd melting behaviour. 170

8.6 (A) TTO-loading within the O1X4-75 polyHIPE estimated gravimetrically under various TTO concentration solution. (B) FTIR spectra of TTO and TTO-loaded porous polyHIPEs at varied TTO loading. (C) Compressional stress-stain curves of O1X4-75 and TTO-loaded T30 polyHIPE measured up to 60% strain. 172

8.7 (A) Cumulative release profiles of TTO-loaded polyHIPEs with varied TTO loading analyzed for 48 h. Inset demonstrate cumulative release profiles of various polyHIPEs during the initial 6 h. (B) Weibull equation fit of various TTO release profiles of TTO-loaded polyHIPEs obtained using Eq. 4. (C) Linear fit of various TTO release profiles obtained using logarithmic form of Ritger-Peppas model in Eq. 5. 175

8.8 (A) Digital images of culture plate demonstrating the CFU in the absence and presence of TTO-loaded polyHIPEs at various TTO concentrations. (B) Bacterial reduction percentage due to TTO-loaded polyHIPEs against *S. aureus* and *E. coli*. (C) Live-dead stained images of *S. aureus* and *E. coli* incubated in presence of pristine and T30 polyHIPE, captured using confocal microscope. (D) TEM images of *S. aureus* and *E. coli* under the influence of pristine and T30 polyHIPE. (E) Absorbance at 260 nm recorded using UV-vis spectrophotometer for *S. aureus* and *E. coli* suspension media treated against pristine and T30 polyHIPE. (F) Biofilm formation by *S. aureus* and *E. coli* observed on the surface of pristine and T30 polyHIPE using FESEM. (G) *S. aureus* and *E. coli* morphologies as seen on pristine and T30 polyHIPE using FESEM. 178

List of Tables

Table number	Table caption	Page number
2.1	Fabrication techniques used to develop scaffolds in various shapes summarized with their basic concept, advantages and limitations.	13
2.2	Various PCL based HIPE templated scaffolds fabricated using preformed polymer, PCL based macromer, or monomer CL stabilized using either surfactant or NP combined with surfactant and polymerized using various comonomers to understand their effect on the properties of final scaffold.	28
6.1	Pickering emulsions stabilized at ϕ_d of 0.55-0.85 using mSiNP and the corresponding emulsion templated nanocomposite scaffolds.	107
6.2	Thermal properties of various non-porous and porous scaffolds during non-isothermal crystallization and melting at 10 °C/min.	114
6.3	Values of Avrami coefficient n, rate constant k_c, and R^2 in various regimes estimated using Avrami plots for non-porous and porous scaffolds.	118
6.4	The values of 2θ, d, and D determined from the deconvolution of XRD patterns of various non-porous and porous scaffolds.	123
7.1	The composition of continuous phase for the preparation of various Pickering HIPE.	133
8.1	Molar ratios of various monomers and crosslinker used for the synthesis of photocured crosslinked PCL.	167
8.2	Values of various constants obtained using Weibull model and Ritger-Peppas model studied for release behaviour of TTO-loaded polyHIPEs.	174

List of symbols

ϕ_d	dispersed phase volume fraction
wt%	weight percentage
°C	degree Celsius
w/v%	weight by volume percentage
g/mol	gram per mole
µm	micrometer
mg/mL	milligram per millilitre
T_g	glass transition temperature
L	litre
g	gram
h	hour
M	molar concentration
mol	number of moles
nm	nanometer
mm	millimetre
kN	kilonewton
min	minute
kV	kilovolt
°C/min	degree Celsius per minute
µL	microlitre
$\mathrm{CFU.mL^{-1}}$	colony forming units per millilitre
rpm	rotations per minute
CO_2	carbon dioxide
M_n	number average molecular weight
$\mathrm{g/cm^3}$	gram per centimetre cube
$\mathrm{mm^2/s}$	millimetre square per second
X_c	crosslink density
M_i	initial weight of sample
M_f	final weight of sample

ρ	density
P	porosity
T_c	crystallization temperature
T_m	melting temperature
ΔH_c	heat of crystallization
ΔH_f	heat of fusion
W_c	relative crystallinity
M_s	swollen sample weight
M_d	dry sample weight
X_{cd}	crystallinity of the sample determined from DSC
X_{cx}	crystallinity of the sample determined from XRD
d	d spacing in XRD
D	crystallite size
X_T	relative crystallinity at temperature T
X_t	relative crystallinity at time t
n	Avrami exponent
k_t	crystallization rate constant in isothermal conditions
k_c	crystallization rate constant in non-isothermal conditions
β	cooling rate in DSC
$t_{1/2}$	crystallization half time
m	Ozawa exponent
$K(T)$	cooling function
ψ	nucleation activity parameter
B^*	nucleation energies of nanocomposite
B	nucleation energies of crosslinked PCL
ΔT_p	supercooling
$F(T)$	fraction of drug released at time *t*
t_{50}	time taken for 50% drug release
Q_t	amount of drug released at time *t*
Q_∞	amount of drug released at time ∞

List of abbreviations

PCL	Poly(ε-caprolactone)
HIPE	High internal phase emulsion
CL	ε-caprolactone
mSiNP	hydrophobically modified fumed silica nanoparticles
CO	clove oil
S. aureus	Staphylococcus aureus
E. coli	Escherichia coli
ECM	extracellular matrix
w/o	water-in-oil
o/w	oil-in-water
o/o	oil-in-oil
HLB	hydrophilic-lipophilic balance
NP	nanoparticles
FDA	Food and Drug Administration
3D	three dimensional
PLA	poly(lactic acid)
MMA	methyl methacrylate
EHA	2-ethylhexyl acrylate
MAAn	methacrylic anhydride
BCY	bis-ε-caprolactone-4-yl
P(VBC-co-DVB)	poly(vinylbenzyl chloride-co-divinylbenzene)
PUU	poly(urethane urea)
semi-IPN	semi-interpenetrating network
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
MSA	methanesulfonic acid
F127	Pluronic F127
polyHIPE	polymerized high internal phase emulsion
ROP	ring opening polymerization
PGA	poly(glycolic acid)

PLGA	poly(lactic-co-glycolic) acid
BMP-2	bone morphogenetic protein 2
VEGF	vascular endothelial growth factor
NaOH	sodium hydroxide
HEMA	hydroxyethyl methacrylate
EGDMA	ethyleneglycol dimethacrylate
$Sn(oct)_2$	Stannous octoate
$CDCl_3$	deuterated chloroform
TPO	5(6)-carboxyfluorescein, trimethylbenzoyldiphenyl phosphine oxide
m-CPBA	3-chloroperbenzoicacid
HCl	hydrochloric acid
MMT	montmorillonite
DMSO	dimethyl sulfoxide
DCM	dichloromethane
NaCl	sodium chloride
KCl	potassium chloride
KH_2PO_4	potassium phosphate monobasic
Na_2HPO_4	sodium phosphate dibasic
$MgSO_4$	anhydrous magnesium sulphate
$NaHSO_3$	sodium bisulfite
$NaHCO_3$	sodium hydrogen carbonate
H_5IO_6	Periodic acid
LB	Luria Bertani broth
DI water	Deionized water
PBS	phosphate buffer solution
FRP	free radical polymerization
TTO	tea tree oil
NMR	Nuclear magnetic resonance
SEM	Scanning electron microscopy
FTIR	Fourier Transform Infrared Spectroscopy
ATR	attenuated total reflection

EDX	Energy-dispersive X-ray spectroscopy
TEM	Transmission electron microscopy
XPS	X-ray photoelectron spectroscopy
DSC	Differential scanning calorimetry
XRD	X-ray diffraction
ZOI	Zone of inhibition
CFU	colony forming units
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
cPCL	crosslinked PCL
EPK	eukaryotic protein kinases
MAPK	mitogen-activated protein kinase
HEC	hydroxyethyl cellulose
ALP	Alkaline phosphatase assay
RB	Reaction buffer
NFATc1	Nuclear factor of activated T cells cytoplasmic 1
RANK	Receptor Activator of Nuclear factor Kappa-B
GFOGER	Gly-Phe-Hyp-Gly-Glu-Arg
DGEA	Asp-Gly-Glu-Ala