FUNCTIONALLY GRADED PLGA-NANO APATITE-LAURIC ACID BIOCOMPOSITE MEMBRANE FOR POTENTIAL CLINICAL APPLICATIONS

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To my dearest mother……..

Mrs. Jannanayagam

For being a mentor, friend and pillar of strength

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ABSTRACT

Bone healing is a challenge in orthopaedics and dentistry. An occlusive membrane is used for the reconstruction of bone defects in guided bone regeneration (GBR) technique. Infection is the major cause for GBR membrane failure in which multiple antibiotics have been used to prevent bacterial colonisation in regenerative clinical practice. An anti-infective membrane with alternative antimicrobial agent to substitute antibiotics is paramount to overcome the incidence of bacterial resistance and side-effects. In this study, a composite membrane was developed by incorporating lauric acid (LA), a naturally derived antimicrobial substance. Poly(lactic-*co*-glycolic acid) (PLGA) based composite membrane was successfully fabricated using a combination of solvent casting-thermally induced phase separation (TIPS)-solvent leaching technique. The triple-layered membrane structure was attained via solvent casting of the composite solutions which then immediately phase separated by freezing at -18±1°C for 24 h. Then, the solvent in phase separated membrane was removed by immersing in precooled water at 3±1°C for 26 h, after which the membrane was air dried at 25°C for 3 days. The triple-layered construct of the PLGA composite membrane was developed with a gradient structure of LA and non-stoichiometric nanoapatite (NAp), to deliver the antimicrobial and osteconductive properties, respectively. The surface morphology and phase composition of the membrane were examined using scanning electron microscopy (SEM) and X-ray diffraction (XRD), respectively. The resulting graded membrane consisted of small pore size layer-1 containing 10wt% NAp + 1-3wt% LA, an intermediate labyrinth layer-2 with 20-50wt% NAp + 1wt% LA, and a large pore size layer-3 containing 30-100wt% NAp without LA. The existence of chemical interaction between PLGA, NAp and LA was identified using Fourier transform infrared spectrophotometry (FTIR) analysis. The synergistic effects of 10-30wt% NAp and 1wt% LA in dry membranes demonstrated higher tensile strength (0.61±0.17 MPa) and elastic modulus (23.15± 6.19 MPa). However, a more pliable behavior with a decrease in elastic modulus (12.50± 4.32MPa) was observed in 3wt% LA added membrane compared to the pure PLGA (20.17±2.21 MPa). The addition of LA resulted in a plasticizing effect at 3wt% due to weak intermolecular interactions in PLGA chains, caused by LA (-OH) and PLGA (C-O) bondings. These results were corroborated by the FTIR peak shift (1-3 cm^{-1}) and glass transition temperature (T_g) reduction as detected using differential scanning calorimeter (DSC). The composite membrane retained its structural integrity with only 22% weight loss after incubation for 24 weeks in phosphate buffered saline (PBS), which indicates its potential use as a physical barrier. The 1-3wt% LA loaded composite membranes had good cell viability toward mouse fibroblasts and showed increased bacterial reduction with increased LA loadings against *S. aureus.* These results demonstrate the potential of LA loaded biocomposite membrane to provide anti-infective surfaces, useful in clinical applications.

ABSTRAK

Penyembuhan tulang adalah satu cabaran dalam bidang ortopedik dan pergigian. Pertumbuhan semula tulang berpandukan (GBR) telah digunakan untuk pembinaan semula kecacatan tulang dengan menggunakan membran penghalang. Jangkitan adalah punca utama kegagalan membran tersebut di mana beberapa antibiotic telah digunakan untuk menghalang pertumbuhan bacteria dalam amalan klinikal. Agen antibakteria alternatif adalah perlu untuk mengatasi kesan sampingan dan rintangan bakteria yang dihasilkan oleh antibiotik. Dalam kajian ini, membran komposit telah dibangunkan melalui penggabungan asid laurik (LA) yang mempunyai sifat antibakteria. Membran komposit berasaskan asid poli(laktik-*co*-glycolic) (PLGA) telah berjaya direka dengan menggunakan gabungan teknik-teknik pelarut tuangan-pemisahan fasa haba teraruh-larut lesap pelarut. Struktur membran tiga-lapis telah dihasilkan melalui pelarut tuangan komposit yang telah melalui pemisahan fasa haba teraruh pada suhu -18±1°C selama 24 jam. Kemudian, pelarut membran telah dibuang dengan merendamkannya dalam air sejuk pada suhu 3±1°C selama 26 jam. Setelah itu, membran telah dikeringkan di udara pada 25°C selama 3 hari. Membran komposit PLGA tiga-lapis ini telah difabrikasi dengan struktur kecerunan melalui penambahan LA dan apatitnano bukan stoikiometrik (NAp) yang memainkan peranan sebagai antimikrob dan penggalak pertumbuhan tulang. Morfologi permukaan dan fasa komposisi membran telah diperiksa dengan menggunakan mikroskopi elektron imbasan (SEM) dan pembelauan sinar-X (XRD). Membran ini terdiri daripada lapisan-1 dengan saiz liang kecil yang mengandungi 10% berat NAp + 1-3% berat LA, lapisan-2 sebagai lapisan perantaraan dengan 20-50% berat NAp + 1% berat LA dan akhirnya lapisan-3 dengan saiz liang besar yang mengandungi 30-100% berat NAp tanpa LA. Kewujudan interaksi kimia antara PLGA, NAp dan LA telah dikenalpasti dengan menggunakan analisis spektrometer inframerah (FTIR). Kesan sinergi diantara 10-30% berat NAp dan 1% berat LA dalam membran komposit kering menunjukkan kekuatan tegangan (0.61± 0.17 MPa) dan modulus elastik (23.15±6.19 MPa) yang tinggi manakala membran mudah bentuk diperolehi dengan penurunan dalam modulus elastik (12.50±4.32 MPa) selepas penambahan 3% berat LA berbanding membran PLGA tulen (20.17±2.21 MPa). Penambahan 3% berat LA mengakibatkan kesan liat disebabkan interaksi lemah dalam rantaian PLGA melalui ikatan LA (-OH) dan PLGA (-CO). Ini telah dibuktikan melalui perubahan puncak FTIR (1-3 cm^{-1}), dan juga penurunan suhu peralihan kaca (T_g) yang dikesan melalui kalorimeter pengimbas kebedaan (DSC). Membran komposit mengekalkan struktur integriti dengan penurunan berat sebanyak 22% selepas rendaman selama 24 minggu di dalam PBS dimana ianya mempunyai potensi sebagai penghalang fizikal. Membran komposit yang mengandungi 1-3% berat LA menunjukkan pertumbuhan sel-sel fibroblas tikus dan juga pengurangan bacteria *S. aureus* dengan peningkatan kandungan LA. Keputusan ini menunjukkan potensi membran komposit yang mengandungi LA sebagai membran anti-jangkitan untuk kegunaan dalam aplikasi klinikal.

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LIST OF SYMBOLS

cfu	-	Colony forming unit
d	-	Interplanar spacing
Exo	-	Exothermic
k	-	drug release kinetic constant
m_0	-	Initial weight
m_1	-	Wet weight
m_2	-	Dry weight
M	-	Molar
M_t	-	Amount of drug released at time t
M_∞	-	Total amount of drug released
n	-	Diffusional exponent
R^2	-	Correlation coefficient
rad	-	Radian
t	-	time
Tg	-	Transition temperature
X_c	-	Crystallinity
X_s	-	Crystallite size
θ	-	Diffraction Angle
λ	-	Wavelength of X-ray beam

LIST OF ABBREVIATIONS

AMP	-	Antimicrobial peptide
ATCC	-	American Type Culture Collection
ASTM	-	American Society for Testing and Materials
ATR	-	Attenuated total reflectance
BET	-	Brunauer – Emmet – Teller
CaP	-	Calcium phosphate
CHN	-	Carbon, Hydrogen, Nitrogen elemental analysis
DSC	-	Differential scanning calorimetry
DDI	-	Double distilled de-ionised
DMSO	-	Dimethyl sulfoxide
DTA	-	Differential thermal analysis
d-PTFE	-	Dense polytetrafluoroethylene
ECACC	-	European Collection of Cell Cultures
e-PTFE	-	expanded PTFE
et al.	-	and others
FDA	-	US Food and Drug Administration
FESEM	-	Field Emission Scanning Electron Microscope
FGM	-	Functionally graded membrane
FTIR	-	Fourier Transform Infrared spectrophotometry
F. nucleatum	-	*Fusobacterium nucleatum*
FWHM	-	Full width at half maximum
GBR	-	Guided bone regeneration
HA	-	Hydroxyapatite
HPLC	-	High Performance Liquid Chromatography
HSF	-	Human Skin Fibroblast cells
ICP-AES	-	Inductively Coupled Plasma-Atomic Emission Spectroscopy
i.e.	-	that is

IC_{80}	-	Inhibition concentration at 80% killing
ICDD	-	International Centre for Diffraction Data
ISO	-	International Organisation for Standardisation
LA	-	Lauric acid
L1	-	Layer 1
L2	-	Layer 2
L3	-	Layer 3
MEM	-	Minimum Essential Medium
MePEG	-	Methoxypoly(ethyleneglycol)
MIC	-	Minimum inhibition concentration
MTT	-	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAp	-	Non-stoichiometric nanoapatite
NApF1	-	Non-stoichiometric nanoapatite Formulation 1
NApF2	-	Non-stoichiometric nanoapatite Formulation 2
NHA	-	Stoichiometric nanohydroxyapatite
OFP	-	Open Flap Debridement
OD	-	Optical density
PBS	-	Phosphate buffered saline
PCL	-	Polycaprolactone
PDL	-	Periodontal ligament
PDLLA	-	poly(DL-lactic) acid
P. gingivalis	-	*Porphyromonas gingivalis*
PGA	-	Polyglycolic acid
P. intermedia	-	*Prevotella intermedia*
PLA	-	Polylactic acid
PLLA	-	poly(L-lactic) acid
PLGC	-	poly (L-lactide-co-glycolide-ε-caprolactone)
PLCL	-	poly (L-lactide-co-ε-caprolactone)
PU	-	Polyetherurethane
PTFE	-	polytetrafluoroethylene
rpm	-	revolution per minute
SEM	-	Scanning Electron Microscopy
SD	-	Standard Deviation

TEM	-	Transmission Electron Microscopy
TGA	-	Thermogravimetric analysis
TIPS	-	Thermally induced phase separation
UV	-	Ultraviolet
UV-Vis	-	Ultraviolet-Visible
wt%	-	Weight percentage
XRD	-	X-ray Diffraction
β-TCP	-	β-tricalcium phosphate
3D	-	3 dimensional

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CHAPTER 1

INTRODUCTION

1.1 Background

Rapid bone defect filling with normal bone is a challenge in the fields of orthopaedic and dentistry [1]. The bone has limited regeneration capability due to insufficient blood supply, large defects and invasion of highly proliferative nonosteogenic tissues that can impair bone repair [2,3]. Bone grafting is an established treatment to restore bone tissue. However, problems such as redundant fibrous connective tissue growth surrounding implanted bone graft and the movement of bone graft particles are still remain to be solved [1]. GBR has become an area of increasing interest in bone restorative procedures for guiding bone healing and regeneration [2,3] due to its success in curing cranial, maxillofacial and alveolar bone defects [4,5]. The concept of GBR is to cover the bone defect using a barrier membrane that enhances new bone ingrowth while preventing the ingrowth of fibrous tissue into the grafted site [6]. Hence, the bone regenerative approaches using GBR membranes have been extensively investigated to reveal their clinical potential [7,8,9].

GBR membranes have been widely studied as they are useful for bone repair in oral and maxillofacial surgery where limited mechanical loading exists [5,10]. The commercially available GBR membranes are made of non-resorbable and resorbable polymers. The non-resorbable polytetrafluoroethylene (PTFE) membranes have exhibited significant disadvantages such as requirement for second surgery and increased risk of infection leading to early removal of the membrane [9].

Collagen based resorbable membranes are widely used in clinical therapies. Since majority collagen membranes are animal derived, these membranes carry the risk of potential transmission of infectious agents, including the inappropriate immune responses in patients [7]. The synthetic resorbable membranes have found widespread use in clinical medicine as they are totally degradable, thus not requiring second surgery [8,9]. Poly(lactic-co-glycolic acid) (PLGA) is a FDA approved synthetic resorbable material and widely used in GBR applications [11,12]. Nonetheless, an inflammatory reaction by the accumulation of acidic degradation products in resorbable membranes has been reported [4,13]. The combination of calcium phosphate (CaP) with resorbable polymeric membranes is expected to neutralize the acidic degradation products from the membranes; which is intended to overcome inflammatory reaction in vivo [13,14,12,15,16]. Moreover, CaP particles in polymeric membranes has been also reported to improve structural integrity, flexibility and bone regeneration in vivo [17,15,18,14]. The aforementioned studies emphasises the need for incorporation of CaP particles to improve physical and mechanical properties of the resorbable polymeric membrane.

Currently, biomaterial-associated infection is regarded as a devastating complication in clinical surgery. Therefore, anti-infective biomaterials need to be developed as the main strategy to prevent infection in clinical applications [19]. A bacteria-free environment is highly important to regenerate bone tissues in GBR strategies [20]. Recently, the antibiotics incorporated GBR membranes have been developed for local delivery of antimicrobial agents [21]. Nonetheless, the increasing bacterial resistance prompted the development of alternative antimicrobial agent incorporated GBR membranes [22,23,20,24]. In light of this, a naturally derived antimicrobial agent to substitute the use of antibiotics is sought after to develop a new antimicrobial membrane for clinical applications.

The antimicrobial properties of naturally found fatty acids have been recognized for many years. Lauric acid (LA) is naturally found in coconut oil [25] and has been recognized to possess broad-spectrum with effective antimicrobial activity against gram-positive bacteria [26,27]. Unlike antibiotics, fatty acids and their derivatives have diverse modes of action that appear to be non-specific and

development of resistance to these compounds has not been reported [28]. It is suggested that LA kills Gram-positive bacteria by separating their inner and outer membranes, resulting in cytoplasmic disorganization of the bacteria [25]. Thus, it is envisaged that incorporating LA in composite membranes for anti-infective bone regeneration purposes could possibly overcome clinical complications caused by the administration of antibiotics.

The development of functionally graded and multiple layered membrane is to enhance the features required for GBR, namely a combination of physical, mechanical, biological and antimicrobial properties [13,23]. Also, the incorporation of functional gradients in a multilayered membrane structure offers the possibilities to overall usefulness to the membrane. Solvent casting technique offers the formation of layered membrane structure [16] whereas porous network formation is attainable through thermally induced phase separation (TIPS) [29] of the polymeric materials. The presence of residual toxic organic solvent is a major concern in solvent based fabrication technique. Thus, it is vital to include solvent removal step to reduce possible toxicity by solvent residues in fabricated membranes [30]. In this study, a new modified solvent casting-TIPS-solvent leaching technique is proposed to fabricate triple layered and graded composite PLGA membrane. Collectively, it is suggested that a new combination of CaP nanoparticles and LA as an antimicrobial agent being graded and layered in PLGA matrices can potentially function as an antimicrobial barrier membrane. This thesis will advance the knowledge in the area of antimicrobial composite membrane development for potential use in cranial, maxillofacial and dental applications. A new technique to establish the fabrication of multilayered and graded composite membrane utilizing solvent casting-TIPS-solvent leaching technique will be developed in this study. The fabrication and structural properties of the triple-layered PLGA membrane, graded with various amounts of LA and CaP nanoapatite will be studied. The effects of LA and CaP addition on the physical, chemical, mechanical, biological and antimicrobial properties of the PLGA composite membrane will also be explored. This membrane will deliver antimicrobial and osteoconductive properties by the incorporation of LA and CaP nanoapatite, respectively.

1.2 Problem statements

The major concerns in GBR surgical intervention are the problems related to the increasing bacterial resistance and side effects caused by antibiotics [31,32]. Multiple antibiotics are currently used to protect the bone defect from bacterial invasion, increasing the risks of bacterial resistance and side effects [33,22,31]. Hence, an alternative antimicrobial agent to substitute antibiotics is sought after. LA has been exhibiting effective antimicrobial activity against gram-positive bacteria that eliminates the need for multiple antibiotics to prevent bacteria colonization [26,27]. Therefore, the incorporation of antimicrobial LA in the composite membrane and its controlled release is proposed to circumvent the above mentioned drawbacks.

Apart from antimicrobial property, other important membrane characteristics such as surface morphology, pore size, membrane degradability, mechanical properties and cytocompatibility should be equally evaluated. Hence, appropriate materials selection and membrane design for GBR applications are highly indispensable for a successful bone defect treatment [7]. Poly(lactic-co-glycolic acid) (PLGA) is a FDA approved synthetic resorbable material which is widely used in GBR applications [11,12]. However, the accumulations of acidic degradation products from the synthetic bioresorbable membranes have been reported to cause inflammatory reaction in vivo [8,9]. Hence, the combination of synthetic polymers with CaP has been reported to neutralize the acidic degradation products from the polymers using ionic interactions [13,14,12,15,16]. Moreover, CaP incorporation improves structural integrity, flexibility and bone regeneration of the resorbable membranes [17,15,18,14]. Therefore, the current clinical disadvantage of using pure synthetic polymeric material as a GBR membrane could be overcome by incorporating CaP particles to reduce the potential inflammatory reactions. Thus, in this study, multiple ions substituted nanoapatite (NAp) powder which has close resemblance to natural bone mineral composition will be synthesized and incorporated into the PLGA matrices to form composite membranes.

Incorporating multiple additives in a composite membrane is a challenge as it requires the development of multilayered and graded membrane structure [13,16,34]. In order to address GBR applications, two functional surface layers are required. One of the surfaces with porous morphology allows bone ingrowth whereas the other dense surface prevents fibrous tissue penetration [16,13]. Therefore, in this study a triple-layered composite membrane with new combination of porous/dense layers will be developed. The NAp particles and LA will be graded in each layer to deliver osteoconductive and antimicrobial properties, respectively.

In order to develop a multilayered and graded composite membrane, an appropriate technique is indispensable to achieve the desired membrane structure. Currently, solvent casting [16] and TIPS [29] techniques have been employed to fabricate composite membranes. However, there are two disadvantages of using solvent casting method: i) toxic organic solvents application [15,18] that requires critical attention especially on its exposure in biomedical applications, ii) CaP particles can spontaneously precipitate from the polymer solution due to poor affinity and can cause non-uniform dispersion of CaP in polymer matrix [18]. Hence, these drawbacks could be overcome by freezing the CaP dispersed polymer matrix structure through TIPS technique. Moreover, solvent removal from the fabricated membrane is another important step to reduce toxic solvent residues [30,35]. Hence, in this study, composite membranes will be fabricated utilizing a new combination of solvent casting-TIPS-solvent leaching technique to address the formation of layered and graded membrane, dispersed with CaP particles and removal of toxic solvent from the membrane. The new modified technique is envisaged to form a composite membrane with graded porous/dense structure that has functional gradients, i.e., NAp and LA.

1.3 Objectives of the study

This work explores a novel fabrication technique, structure and design of a polymer-ceramic composite membrane incorporating LA as an antimicrobial agent. The goal is to design a functionally graded triple layered barrier membrane with

antimicrobial property using solvent casting-TIPS-solvent leaching techniques. In order to achieve the main objective, the following specific objectives were executed.

a) To synthesise multiple ions substituted non-stoichiometric nanoapatite (NAp) powder.
b) To establish a combined solvent casting-TIPS-solvent leaching techniques for the formation of triple-layered PLGA composite membranes graded with LA and NAp powder.
c) To determine the physical, chemical, mechanical and in vitro degradation properties of the membrane.
d) To evaluate the cytocompatibility and antimicrobial efficacy of the membrane.

1.4 Research hypothesis

It is possible to achieve an antimicrobial composite membrane by incorporating antimicrobial agents, in order to prevent biomaterial-associated infection in GBR applications. Therefore, it is envisaged that incorporating LA in the composite membrane could impart antimicrobial property which could prevent bacterial infection associated to the membrane. Furthermore, a resorbable composite membrane is desired to achieve less in vivo inflammation by reducing acidic degradation products through the addition of CaP particles [8,9]. Moreover, the combination of synthetic resorbable membranes with CaP is expected to deliver improved mechanical strength to the composite membranes [17,15,18,14]. Hence, in this study, it is hypothesised that varying the NAp and LA contents in PLGA matrices can significantly alter the physico-chemical, mechanical and antimicrobial properties of the membrane.

The GBR membrane is designed to have a smooth surface on one face to inhibit soft tissue penetration while the opposite porous face is capable of accommodating bone tissue ingrowth in vivo [16,36]. The dense/porous network formation through TIPS [29] technique is easily attainable whereas a multilayered membrane structure via solvent casting and the removal of solvent [30] could translate a safer membrane fabrication technique for clinical practice. The solvent

casting-TIPS-solvent leaching technique will be used to test the hypothesis that one can tailor the properties of the different layers to form a functionally graded composite membrane to retain its structural, dimensional and mechanical properties for bone regeneration. Figure 1.1 demonstrates the importance of incorporating LA in composite membrane which may prevent bacterial infection on the membrane surface. In addition, formation of dense membrane surface also excludes fibroblast penetration into the barrier membrane.

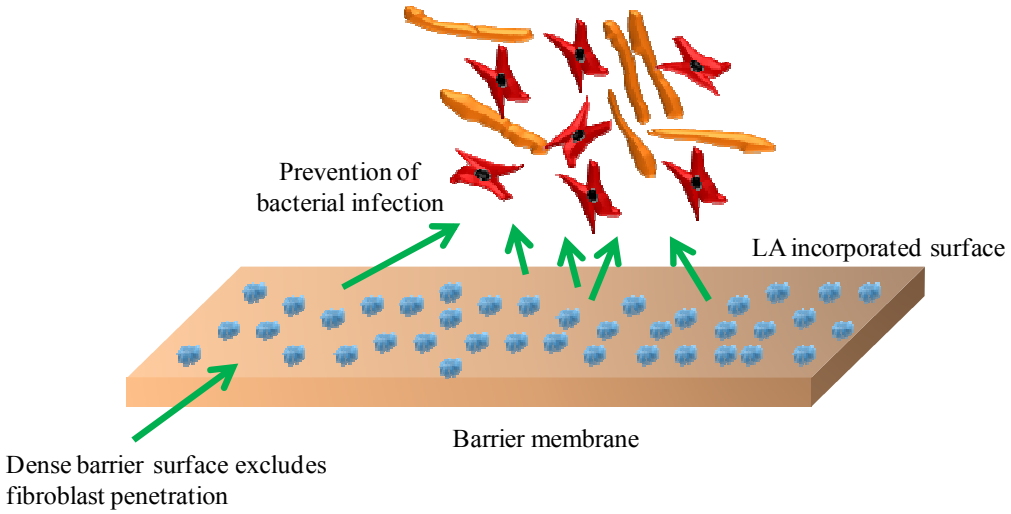

Figure 1.1: LA incorporation into barrier membrane as an antimicrobial agent for adjunct treatment in GBR procedures to inhibit bacterial infection.

1.5 Scope of the study

A new design of triple-layered and graded PLGA composite membrane has been fabricated. The triple layered membrane is comprised of PLGA matrix, graded with non-stoichiometric NAp and LA at each layer. PLGA with a lactic acid to glycolic acid ratio of 85:15 degrade over 2–6 months [37] and have the ability to deliver drugs locally in a controlled manner. These properties are making it suitable for use as a GBR barrier membrane. Besides improving mechanical strength of the membranes, the incorporation of CaP particles should be merely targeted for its

osteoconductivity and hydrophilic nature to enhance bone growth into the polymer surfaces [38]. NAp powder is synthesized by introducing substituents within 1.84wt% (Na), 1.46wt% (Mg), 0.06wt% (K) and 4.80wt% (CO_3^{2-}) to closely mimic natural bone apatite. The NAp powder is incorporated to enhance bioactivity and osteoconductivity of the membrane. LA is added to introduce antimicrobial properties to the composite membrane to prevent bacterial infection as it is known to possess effective antimicrobial activity against gram-positive bacteria [26,27]. The composite membrane is fabricated by employing a modified solvent casting-TIPS-solvent leaching technique. The solvent casting facilitated lamination of multiple layers of graded LA and NAp in PLGA matrices whereas TIPS used to form porous/dense layers in the membrane structure. Solvent leaching is performed to remove toxic solvent residues.

1.6 Significance of the study

LA, as a substitute for antibiotics is identified and incorporated in the composite membrane which is to be used as a potential antimicrobial membrane for clinical applications. Prevention of bacterial infection is a promising strategy whereby LA imparts antimicrobial activity on the membrane surface. This would render an antimicrobial barrier membrane appropriate for adjunctive treatment in guiding bone regeneration. This work also reports the fabrication of PLGA-NAp-LA composite membrane using solvent casting-TIPS-solvent leaching technique. This new technique largely eliminates the solvent residue in the fabricated membrane through solvent leaching step using water as the exchanging medium.

1.7 Thesis outline

Chapter 1 is the introduction to the study of this thesis. The entire outline of the thesis is illustrated in Fig. 1.2.

Chapter 2 describes the review of literatures related to the development and application of commercially available GBR membrane that has been related to its profound improvement through current research to overcome clinically reported shortcomings. Moreover, selection criteria for PLGA, LA and NAp are also reviewed to ensure the fabricated composite membrane is more likely to possess appropriate physical, structural, dimensional, mechanical, antimicrobial and biological properties for potential use in bone regeneration procedures.

Chapter 3 deals with the materials and methods used to investigate the appropriate parameters, experimental set-up, test conditions, characterization using analytical equipment and material evaluation involved in the fabrication and evaluation of the composite membranes. The synthesis of NAp powder is reported in the first part of the chapter. Subsequently, the development of PLGA based NAp-LA composite membrane through a new fabrication technique using solvent casting-TIPS-solvent leaching is reported. This is followed by the development of methods to test on the membrane's properties such as physico-chemical, mechanical, in vitro degradation profile over six months duration, quantification of LA release and finally, LA release mechanism; since the effects of NAp and LA additions in the PLGA membranes are highly imperative to meet the design criteria of membranes for GBR applications.

Chapter 4 elaborates the outcome of NAp synthesis, fabrication of composite membranes, degradation profiles for composite membranes, mechanical evaluation of membranes in dry and wet condition, released LA concentration and its release mechanism. Synthesis of NAp with the highest substitutent composition, the morphology of triple layered membrane, phase composition, physical changes in amorphous/crystalline state of LA, interaction mechanisms between PLGA-NAp-LA in composite membranes, weight loss and water absorption of membranes, and finally the quantification of LA release and its release mechanism from composite membranes for sufficient antimicrobial effects while maintaining its cytocompatibility are discussed. The cytocompatibility of synthesized NAp powder and composite membranes along with antimicrobial evaluation on the effects of LA addition in composite membranes were discussed.

Chapter 5 concludes structural, dimensional and mechanical integrity of the layered and graded composite membrane. The effects of LA and NAp addition on physico-chemical, mechanical and antimicrobial properties are also described.

Publications and presentations at conferences: This section forms part of the thesis, which described the synthesis of NAp powder and the fabrication of composite membranes published in peer reviewed impact factor journals and presented at international conferences as listed in Appendix A.

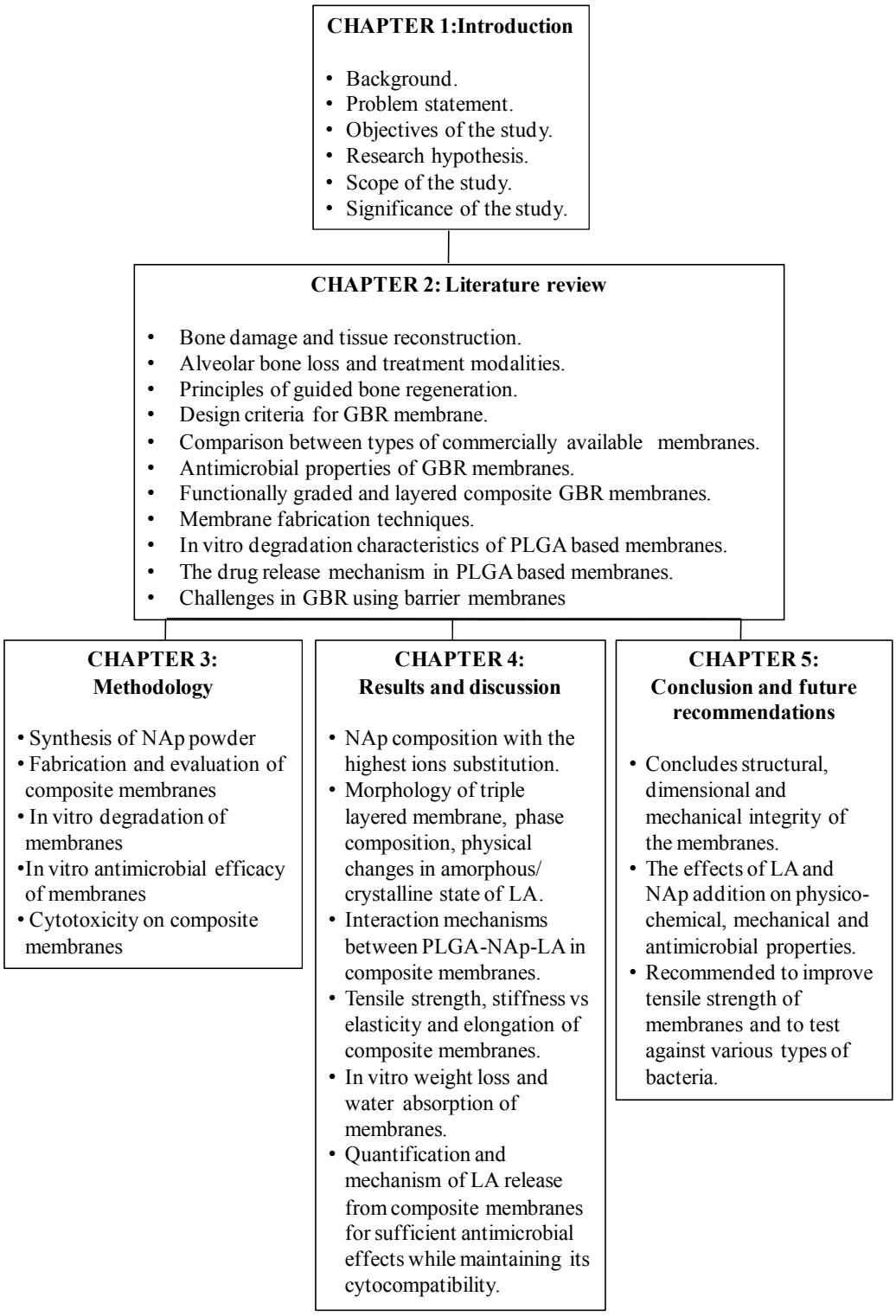

Figure 1.2: Representation of thesis outline.

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