

ANTIBACTERIAL AND CYTOTOXICITY ANALYSES ON TRIPLE
LAYERED POLY(LACTIC-CO-GLYCOLIC ACID)/NANOAPATITE/LAURIC
ACID COMPOSITE MEMBRANE

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MEMBRANE

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ABSTRACT

Guided tissue regeneration (GTR) membrane has been extensively used for repair and regeneration of damaged periodontal tissues. It acts as a barrier to prevent down-growth of epithelial and connective tissues into the defects, thus allowing periodontal regeneration. Current commercial GTR membranes are susceptible to bacterial colonization, leading to premature membrane degradation. The purpose of this research was to prepare GTR membranes with antibacterial and biocompatibility properties. The triple layered composite membranes consisted of poly(lactic-co-glycolic acid) (PLGA) and lauric acid (LA) substituted nanoapatite (NAp) were fabricated using solvent casting and thermally induced phase separation/solvent leaching technique. The physical properties of PLGA/NAp/LA membrane were measured by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Antibacterial effect of the composite membranes (1, 2 and 3 wt% LA) was then investigated on *Phorphyromonas gingivalis* and *Fusobacterium nucleatum* through disc-diffusion and percent reduction tests. MTT cell culture tests were conducted to evaluate the effects on the cells viability. Significantly, these composite membranes exhibited patterns of inhibition and killing effect against both periodontal microorganisms. Increase in LA content tended to increase the bactericidal activity. The PLGA/NAp/LA composite membranes possessed good biocompatibility by demonstrating positive effects on the cell morphology, viability and proliferation. Therefore, the PLGA/NAp/LA composite membranes can be classified as a prospective biodegradable GTR membrane for future periodontal application.

ABSTRAK

Membran pertumbuhan tisu berpandu (PSTB) telah digunakan secara meluas untuk memperbaiki dan menumbuhkan tisu periodontal yang rosak. Ia bertindak sebagai sempadan yang mencegah pertumbuhan ke bawah tisu epitelial dan penghubung ke tempat kerosakan, lalu membenarkan pertumbuhan periodontal. Membran PSTB masa kini cenderung dijangkiti pengkolonian bacteria, mengarah kepada penguraian membran pramatang. Tujuan kajian ini adalah untuk menyediakan membran PSTB dengan ciri antibakteria dan biokeserasian. Membran komposit tiga lapisan terdiri daripada asid poli (laktik-*ko*-glikolik) (PLGA) dan asid laurik (LA) menggantikan apatit nano (NAp) diperbuat menggunakan teknik acuan larutan dan pemanasan yang mengaruhkan fasa pengasingan/larut lesap larutan. Ciri-ciri fizikal membran PLGA/NAp/LA diukur dengan spektroskopi inframerah transformasi Fourier (FTIR) dan mikroskopi pengimbas elektron (SEM). Kesan antibakteria membran komposit (1, 2 dan 3 wt% LA) telah dikaji ke atas bakteria *Phorphyromonas gingivalis* dan *Fusobacterium nucleatum* melalui kaedah cakera resapan dan peratusan pengurangan. Pengasaan kultur sel MTT dijalankan untuk menilai kesan terhadap kebolehidupan sel. Signifikasinya, membran komposit ini menunjukkan corak perencatan dan kesan pembunuhan terhadap kedua-dua mikroorganisma periodontal. Peningkatan kandungan LA telah meningkatkan aktiviti bakterisidal. Membran komposit PLGA/NAp/LA mempunyai biokeserasian yang baik dengan menunjukkan kesan positif ke atas morfologi sel, kebolehidupan, dan proliferasi. Oleh itu, membrane komposit PLGA/NAp/LA boleh dikelaskan sebagai membran penguraian PSTB yang berprospektif untuk aplikasi periodontal di masa hadapan.

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LIST OF ABBREVIATIONS

<i>GTR</i>	-	Guided tissue regeneration
<i>TCH</i>	-	Tetracyclin hydrochloride
<i>LA</i>	-	Lauric acid
<i>PLGA</i>	-	Poly(lactic- <i>co</i> -glycolic acid)
<i>NAp</i>	-	Nanoapatite
<i>TIPS</i>	-	Thermally induced phase separation
<i>FESEM</i>	-	Field emission scanning electron microscopy
<i>FTIR</i>	-	Fourier transform infrared spectroscopy
<i>MTT</i>	-	3(-4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
<i>PDL</i>	-	Periodontal ligament
<i>E-PTFE</i>	-	Expanded polytetrafluoroethylene
<i>PLA</i>	-	Poly(lactic acid)
<i>PGA</i>	-	Poly(glycolic acid)
<i>PCL</i>	-	Poly(caprolactone)
<i>SLS</i>	-	Surface layer
<i>CL</i>	-	Core layer
<i>FFA</i>	-	Free fatty acids
<i>AMPS</i>	-	Antimicrobial peptides
<i>LPS</i>	-	Lipopolysachharide
<i>DMSO</i>	-	Dimethyl sulfoxide
<i>DMEM</i>	-	Dulbecco's modified eagles medium
<i>PBS</i>	-	Phosphate buffered saline
<i>EDTA</i>	-	Ethylenediaminetetraacetic acid

<i>HSF</i>	-	Human skin fibroblast
<i>ATCC</i>	-	American Type Culture Collection
<i>ATR</i>	-	Attenuated total reflectance
<i>BUARL</i>	-	Balai Ungku Aziz Research Laboratory
<i>ASTM</i>	-	American for Testing and Materials

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

In dental practice, guided tissue regeneration (GTR) membrane is a well-established therapy in the treatment of mandible and alveolar bone defects that is infected by periodontal disease [1-2]. The concept of GTR is to act as a barrier in preventing the down-growth of epithelial and connective tissues into the defect [2]. Therefore, the defects will provide a medium for periodontal regeneration without the interruption of other tissues [3]. A synthetic resorbable membrane is widely used in the application of clinical medicine [2-5]. However, an inflammatory reaction by the accumulation of acidic degradation products in the polylactic acid membranes has been reported [4-7]. These significant disadvantages presented by the previous GTR membranes demonstrate that the “ideal” periodontal membrane for periodontal regenerative therapy is not yet to be found [1-2].

Several periodontal pathogens are responsible for the failure of bone regeneration process [3-4]. Indeed, the presence of periodontal pathogens such as *Porphyromonas gingivalis* (*P. gingivalis*) may affect the success of periodontal

regeneration [8]. Machtei *et al.* suggested that, periodontal pathogens should be controlled in the site of membrane insertion in order to ensure a successful regeneration [8]. Therefore, it is extremely paramount to control and reduce bacterial contamination on the membrane in order to enhance periodontal regeneration [1]. Several antibiotics and antibacterial agents have been used extensively to overcome this problem [9]. Multiple researchers have successfully incorporated tetracycline hydrochloride (TCH) and metronidazole benzoate into different polymeric solutions, with the aim in developing a material for therapeutic purpose [2-3,10]. However, there are very few studies which explored the incorporation of antibacterial agents into the GTR membrane [5].

Lauric acid (LA) is one of the typical free fatty acids found in human sebum and natural products such as coconut palm and milk [10]. It has strong antimicrobial activity while not inducing any cytotoxicity effect to human sebocytes [10]. Lauric acid, an amphiphilic molecules, consists of hydrophobic hydrocarbon [11] chain and hydrophilic carboxylic acid head group, which makes it suitable for antibacterial application [10-12]. Furthermore, it has the greatest antimicrobial activity among all medium chain aliphatic fatty acids [12]. The mechanism by which this lipid kills bacteria has been reported where previous microscopy studies demonstrated that the lipid disrupted bacterial cell membrane [13].

Although LA exerts strong antimicrobial activity against many microorganisms, it is still unknown if it has similar effect on the periodontal therapy or whether it can be used as a natural antimicrobial agent in the GTR membrane [13]. Therefore, this study aimed to determine the antibacterial efficacy and cytocompatibility of the recently developed functionally-graded GTR membrane composed of poly(lactic-*co*-glycolic acid) (PLGA), nanoapatite (NAP) and LA. The percentage of each material was controlled to provide an optimum antibacterial effect without causing the cells to dysfunction.

1.2 Problem Statement

Guided tissue regeneration membrane is a well-established therapy in the treatment of mandible and alveolar bone defects [14]. However, there are several problems and limitations which may arise following the restoration of GTR membrane such as inflammation reaction occurs due to accumulation of acidic degradation products from the resorbable membrane [94]. The membrane function in assisting periodontal regeneration is deteriorated by the presence of periodontal pathogens such as *P. gingivalis*, *Fusobacterium nucleatum* (*F. nucleatum*) and *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) [15]. In order to protect the periodontal defect from bacterial invasion, multiple antibiotics are currently used, thus increasing the risks of bacterial resistance and side effects. Problems concerned over bacterial resistance and side effects by the systemic administration and localized release of antibiotics cannot be ignored in GTR surgical intervention [8]. None have reported about the incorporation of the antibacterial properties of lauric acid into the GTR membrane. Therefore, this work will investigate the potential of lauric acid as the naturally derived antimicrobial agent in GTR barrier membrane.

1.3 Objectives of the Study

1. To prepare and characterize the developed functionally-graded GTR membrane composed of PLGA, NAp and LA.
2. To determine the antibacterial properties of the membrane against *P. gingivalis* and *F. nucleatum*.
3. To determine the cytocompatibility of the membrane towards fibroblast cells.

1.4 Scope of the Research

Functionally-graded GTR membranes composed of PLGA, NAp and LA were prepared using thermally induced phase separation (TIPS) and solvent leaching techniques. The

membranes were characterized by using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Antibacterial properties of the membranes were investigated against two types of bacteria: *P. gingivalis* and *F. nucleatum*. Cytocompatibility of the membranes was assessed by conducting MTT assays on fibroblast cells.

1.5 Significance of the Study

The prepared functionally-graded GTR membrane is able to address the current problems in the treatment of mandible and alveolar bone defects caused by periodontal diseases. The three functionally-graded layers is an effective barrier function that meets the unique needs of hard and soft tissues. Inflammatory reaction due to the formation of excessive degradation product is therefore very unlikely. The addition of NAp on the bone-sided layer can greatly enhance bone regeneration process. The incorporation of LA into the soft-tissue-sided layer will selectively target and kill periodontal bacteria. The use of natural derived LA will eliminate the disadvantage of bacterial resistance from antibiotic. The developed functionally-graded GTR membrane will be subjected to a patent filling once its efficacy is scientifically proven.

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APPENDIX

Appendix A Triple layered membrane