

## TABLE OF CONTENTS

	Page
Acknowledgement	v
iii Abstract (English)	viii
Abstract (Thai)	xi
Table of Contents	xiv
List of Tables	xv
List of Figures	xviii
Abbreviations	1
Chapter 1 Introduction	1
1.1 Principles, theory and rationale	5
1.2 Objectives	8
1.3 Hypotheses	6
1.4 Education/application advantage	7
1.5 Research designs, scope and methods	10
Chapter 2 Literature review	10
2.1 Immunopathogenesis of periodontal disease	12
2.2 Human gingival epithelial cells (HGECs)	13
2.3 Human gingival fibroblasts (HGFs)	16
2.4 Matrix metalloproteinases (MMPs)	16

2.5 <i>Fusobacteriumnucleatum</i> ( <i>F. nucleatum</i> )	26
2.6 Phospholipase D (PLD)	29
2.7 Phospholipase A <sub>2</sub> (PLA <sub>2</sub> )	33
Chapter 3 Materials and Methods	39
3.1 Materials	39
3.2 Cultures of human gingival epithelial cells	40
3.3 Cultures of human gingival fibroblasts	41
3.4 Cell stimulation	41
3.5 Isolation of total RNA and RT-PCR	42
3.6 Real-time PCR	46
3.7 Western blot analyses for cytoplasmic and nuclear extracts and culture supernatants	46
3.8 Gelatin zymography and quantification of gelatinolytic area	48
3.9 Immunofluorescence	49
3.10 Assays for PLD activity	49
3.11 Extraction and thin-layer chromatography of lipids	50
3.12 Statistical analysis	51
Chapter 4 Results	52
4.1 Expression and activity of MMP -9 and MMP-2 in human gingival epithelial cells	53
4.2 Constitutive expression of MMP-2 in human gingival fibroblasts	62

4.3 Expression of PLD1 and PLD2 mRNA and protein and their activity in human gingival epithelial cells	68
4.4 Involvement of PLD enzymes in induction of MMP-9 expression and secretion	75
4.5 Induction of MMP-9 expression and secretion by dioctanoylphosphatidic acid	79
4.6 Cytosolic phospholipase A <sub>2</sub> $\alpha$ is constitutively expressed, but can be transiently activated by phosphorylation	87
4.7 Induction of MMP-9 expression and activity is controlled by cPLA <sub>2</sub> $\alpha$	93
Chapter 5 Discussion	97
References	107
Appendices	133
Appendix A Certificate of Ethical Clearance	134
Appendix B Inform consent	135
Appendix C Bacterial crude cell wall preparation	136
Curriculum Vitae	137

**LIST OF TABLES**

Table	Page
2.1 A family of human MMPs and their substrate specificities	18-19
3.1 The summary of the sequences of primers and the amplicon sizes in base pairs (bp)	44
3.2 The summary of amplification conditions for each gene	45

## LIST OF FIGURES

Figure	Page
2.1 The domain structure of MMPs	20
2.2 A model of proMMP-2 activation by MT1-MMP and TIMP-2	24
2.3 Phospholipase D-catalyzed reactions	31
2.4 Phospholipase A <sub>2</sub> on the metabolic pathway of lipid mediator production	34
2.5 The four paralogs of the Group IV cPLA <sub>2</sub>	36
2.6 A schematic diagram shows the importance of tyrosine phosphorylation and the involvement of cPLA <sub>2</sub> in MMP-9 up-regulation in human monocyte	38
4.1 Up-regulation of MMP-9 mRNA in HGECs	55
4.2 Real-time PCR assay of MMP-9 mRNA expression	56
4.3 MMP-9 protein induction in HGECs	57
4.4 Induction of MMP-9 activity in HGECs	58
4.5 The time-course study shows an early MMP-9 mRNA induction	59
4.6 Late MMP-9 protein secretion in HGECs	60
4.7 Late MMP-9 activity in HGECs	61
4.8 Constitutive expression of MMP-2 in HGFs	64
4.9 The time-course study demonstrates constitutive MMP-2 mRNA expression	65
4.10 The expression of MMP-2 protein and its activity in HGFs	66
4.11 Induction of MMP-2 activity in treated and untreated HGFs	67
4.12 Expression of PLD1 and PLD2 mRNA in HGECs by Western Blot analysis	70

4.13	Densitometric analyses of mRNA expression for two PLD1 splice variants and PLD2 in HGECs	71
4.14	Expression of PLD1 and PLD2 protein in HGECs	72
4.15	An assay for PLD activity in HGECs	73
4.16	Thin-layer chromatogram (TLC) shows the time course study of phosphatidic acid (PA) formation in HGECs	74
4.17	Dose-dependent inhibition of MMP-9 mRNA induction by the PLD inhibitors, including ethanol and 1-butanol	76
4.18	Dose-dependent inhibition of induced MMP-9 activity by the PLD inhibitors	77
4.19	Densitometric analyses of the gelatinolytic activities of MMP-9	78
4.20	Up-regulation of MMP-9 mRNA by DOPA in HGECs by RT-PCR analysis	80
4.21	Up-regulation of MMP-9 protein by DOPA in HGECs by Western blot analysis	81
4.22	Up-regulation of MMP-9 activity by DOPA in HGECs by Gelatin zymography	82
4.23	Inhibition of MMP-9 mRNA induction by the PAP inhibitor, propranolol	83
4.24	Inhibition of induced MMP-9 activity by propranolol	84
4.25	Up-regulation of MMP-9 mRNA by DOG in HGECs by RT-PCR analysis	85
4.26	Up-regulation of MMP-9 activity by DOG in HGECs	86
4.27	Constitutive mRNA expression of cPLA <sub>2</sub> α	89
4.28	Transient activation of cPLA <sub>2</sub> by phosphorylation	90
4.29	Nuclear localization of the phosphorylated form of cPLA <sub>2</sub>	91
4.30	Transient activation of cPLA <sub>2</sub> by phosphorylation, cytoplasmic and nuclear extraction	92
4.31	Involvement of cPLA <sub>2</sub> α in induction of MMP-9 mRNA expression	94

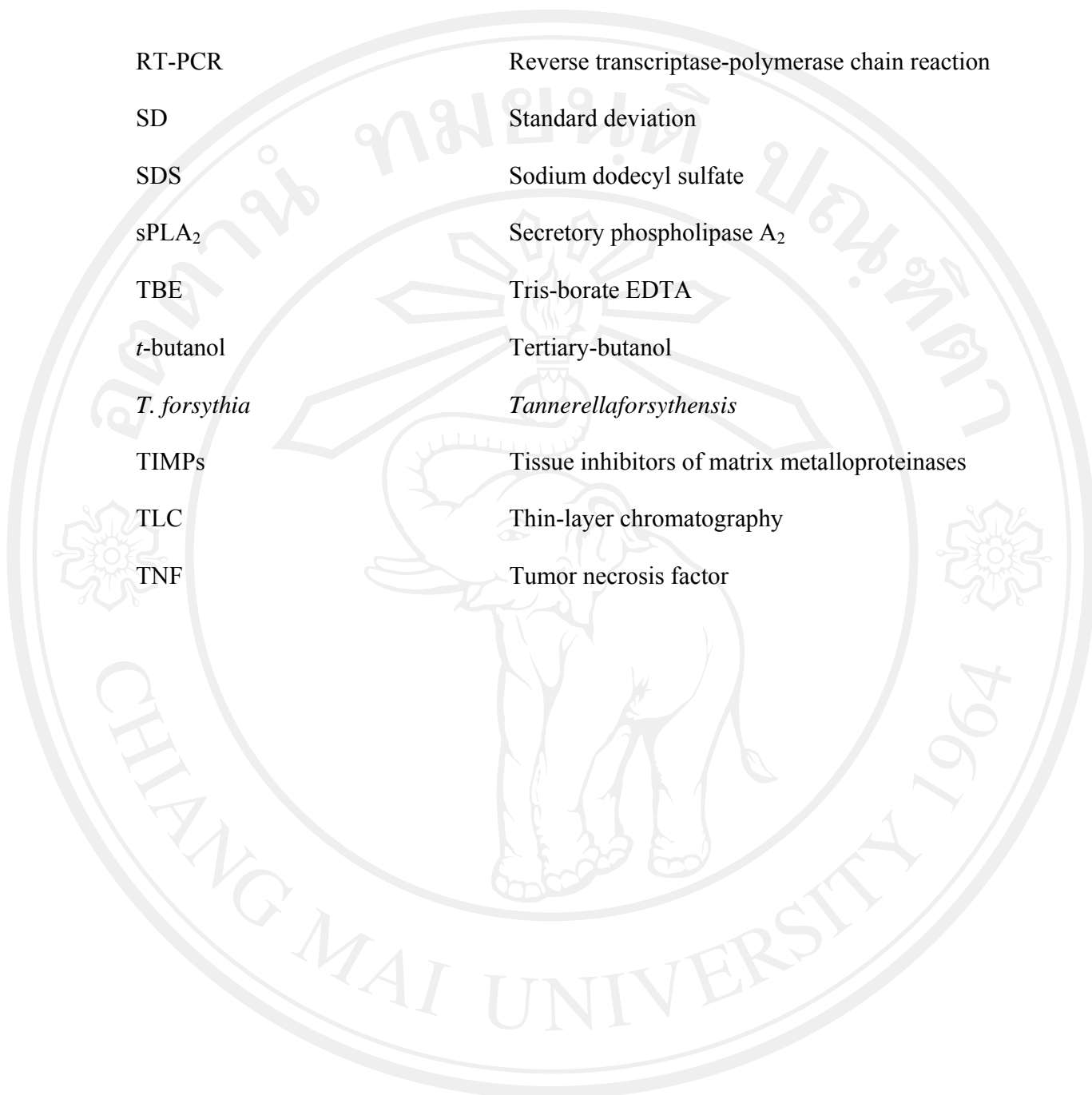
4.32	Involvement of cPLA <sub>2</sub> α in induction of MMP-9 activity	95
4.33	The percentage of MMP-9 mRNA inhibition as expressed by the expression of MMP-9 relative to GAPDH	96
5.1	A schematic diagram shows the importance of PLD and cPLA <sub>2</sub> α in MMP-9 up-regulation in HGECs	106

### LIST OF ABBREVIATIONS

AA	Arachidonic acid
<i>A. actinomycetemcomitans</i>	<i>Aggregatibacter actinomycetemcomitans</i>
ARF	ADP ribosylation factor
BM	Basement membrane
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CCD	Charge-coupled device
Con A	Concanavalin A
COX	Cyclooxygenase
DOG	Diocanoyl glycerol
DOPA	Diocanoylphosphatidic acid
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
DTT	Dithiothreitol
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
FITC	Fluorescein
<i>F. nucleatum</i>	<i>Fusobacterium nucleatum</i>
GCF	Gingival crevicular fluid
hBD-2	Human $\beta$ -defensin-2
HGECs	Human gingival epithelial cells



HGFs	Human gingival fibroblasts
IL	Interleukin
IFN	Interferon
KGM	Keratinocyte growth medium
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
MMPs	Matrix metalloproteinases
mRNA	Messenger ribonucleic acid
MT-MMPs	Membrane type- matrix metalloproteinases
PA	Phosphatidic acid
PAF	Platelet activating factor
PBS	Phosphate buffer saline
PBut	Phosphatidylbutanol
PC	Phosphatidylcholine
PEt	Phosphatidylethanol
PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
<i>P. gingivalis</i>	<i>Porphyromonasgingivalis</i>
PIP <sub>2</sub>	Phosphatidylinositol 4,5-bisphosphate
PIP <sub>3</sub>	Phosphatidylinositol 3,4,5-triphosphate
PKC	Protein kinase C
PLD	Phospholipase D
PLA <sub>2</sub>	Phospholipase A <sub>2</sub>
PMA	Phorbol 12-myristate 13-acetate



RT-PCR	Reverse transcriptase-polymerase chain reaction
SD	Standard deviation
SDS	Sodium dodecyl sulfate
sPLA <sub>2</sub>	Secretory phospholipase A <sub>2</sub>
TBE	Tris-borate EDTA
<i>t</i> -butanol	Tertiary-butanol
<i>T. forsythia</i>	<i>Tannerella forsythensis</i>
TIMPs	Tissue inhibitors of matrix metalloproteinases
TLC	Thin-layer chromatography
TNF	Tumor necrosis factor